# MICROSCOPIC TECHNIQUE
## *in*
# BIOLOGY *and* MEDICINE

BY

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# PREFACE

What appeared altogether impossible twenty-five years ago has in several cases been attained by improvements in technique. Who would have believed at that time that ultramicroscopes would now be manufactured in quantity, built without any optical lenses, and capable of revealing a world of structures quite beyond their ken? Who would have thought that a whole series of different atoms could be tagged and their distribution to the several tissues, when introduced into the body, accurately measured? Who would have anticipated the significant and unexpected new developments which have been made in polarization optical methods? Had we been told twenty-five years ago that the cell itself can be broken up into parts several of which can be collected in quantity and chemically analyzed, we would have been incredulous. All this and more has been achieved as a result of team work between the biological and physical sciences. And we may believe that more surprises are in store.

Yet some of us individually are still extraordinarily conservative in the methods we use. The possibilities of improving old techniques, of replacing some of them by new ones and of relying more upon microchemical and physical procedures are not explored as they should be. The purpose of this book is to extend the horizon by exposing in an introductory way a few of the many opportunities awaiting workers in biology and medicine interested in the minute structure of living things. Success will depend upon ability to anticipate and meet the needs of those likely to consult it. Definite information about specific matters is likely to be more in demand than general statements. The latter are limited to a few pages and deal with "choice of methods" and "organization of laboratory."

Some may turn to the names of the structures in which they happen to be most interested at the moment—**Nissl Bodies, Nerve Fibers, Capillaries** and so forth—on the off chance of finding some useful hints as to methods better adapted for their microscopic study, the most likely experimental errors and so on. Because the range of cells, parts of cells, tissues, organs and systems is obviously so immense, mention is only possible of a small proportion of them so that much depends on the selection made.

Others may seek information under the headings of elements such as **Iron, Potassium** and **Calcium,** of enzymes like **Pepsin** and **Phosphatase** and of many other components of living material. It is difficult to draw the line but most of those that can be localized microscopically are mentioned, likewise techniques for the determination of permeability, viscosity, pH and other properties of tissues.

It often happens, however, that data are required about a particular technique, which the workers are using or expect to use, and which is known to them by the names of those who discovered it, as for example the methods of **Giemsa** and

**Mallory.** Consequently information also must be supplied under various names though this is usually less satisfactory than under subjects. A very annoying handicap is the host of synonyms for dyes. Being ignorant of chemistry, I have with confidence listed those given by Dr. H. J. Conn. Many more will be found in The Colour Index of the Society of Dyers and Colourists.

Since all are busy people, time is a factor and they will wish to dig out what they want as directly and quickly as possible. It is for this reason that everything is listed alphabetically. Obviously this book can be nothing more than a brief entré to microscopic technique. Therefore, numerous references to the literature are supplied for follow up. Again to save time, these are given each in its appropriate place, thus avoiding the necessity of turning the pages and locating them in a large bibliography. But no attempt is made to trace the techniques to their original exponents and to apportion credit for numerous modifications. Often the most recent and accessible reference is provided relying on the author to state history fairly. Evidently, in order to keep up to date as to methods, the reader must repeatedly consult the latest issues of many journals. Stain Techn.; J. Lab. & Clin. Med.; J. Tech. Meth.; Bull. d'Hist. Appl.; and Zeit. f. mikr. Tech. are particularly valuable.

Finally I wish to thank my colleagues for their help, particularly Drs. L. R. Boling, C. Carruthers, William Cramer, Morris Moore, J. L. O'Leary, W. L. Simpson, R. E. Stowell, Lester Wicks and Dr. H. J. Conn, Chairman of the Biological Stain Commission, who very kindly read the manuscript and made several useful suggestions.

## CHOICE OF METHODS

The selection will depend upon what it is desired to do. In most cases a particular kind of information is sought. Feasible methods of obtaining it with the materials available are needed and it is important that the information secured be trustworthy having a minimum of experimental error. A brief outline of what can be done is presented in the hope that some of the techniques mentioned will be suitable or will suggest satisfactory ways to proceed. Further data are given in the body of this book concerning the subjects given in bold face type.

### 1. To Examine Directly in Vivo

The ideal arrangement is to look into the body and to study its parts as they function without causing any disturbance. With protozoa and certain small transparent invertebrates this is relatively simple. The web of a frog's foot is thin and can easily be looked through without seriously interfering with the frog. Some other parts of the bodies of various aquatic lower forms lend themselves to direct examination *in vivo*; but there are definite limitations in such a study of what is going on in the human body. It is possible to peer into the various apertures but to get close enough to the living tissues to use high magnifications is not feasible. The cornea and lens of the eye are transparent and much valuable information can be secured by direct examination of the retinal blood vessels. Even here their distance from the surface is considerable and magnification is therefore limited. As far as we know at present the best that can be done is to take advantage of a discovery, made by Lombard (W. P., Am. J. Physiol., 1911–12, **29,** 335–362) that the epidermis can be rendered transparent by the addition of a little highly refractile oil without noticeably injuring it or disturbing the underlying tissues. By this means the blood vessels of the dermal papillae in the fold of skin over the nail bed, which are very near to the surface, can be studied directly at fairly high magnification and over long periods of time thus permitting the making of excellent pictures. See review of literature by Wright, I. S. and Duryee, A. W., Arch. Int. Med., 1933, **52,** 545–575.

That the lymphatics in the human skin can be made visible *in vivo* by the injection of small amounts of Patent Blue V has been demonstrated by Hudack, S. S. and McMaster, P. D., J. Exp. Med., 1933, **57,** 751–774. The vessels in the ears of living mice can readily be seen without any surgical procedure. It is even possible to directly watch the dye, Chicago blue, after intravenous injection elsewhere in the body, leak out into the tissues especially through the walls of the venules (Smith, F. and Rous, P., J. Exp. Med., 1931, **54,** 499–514). Ideas as to the relative hydrogen ion concentrations of some of the tissues visible from without can be secured by the injection of **Hydrogen Ion Indicators** (Rous, P., J. Exp. Med., 1925, **41,** 739–759). The opportunities are many especially in animal experimentation.

## 2. To Examine through Windows in Vivo

The construction of windows in the skin or body wall through which the tissues can be examined in vivo is a less ideal technique because it involves surgical interference with the body. In the most used of these techniques a hole is made through a rabbit's ear from one surface to the other. A glass chamber is then sewed into the hole in such a way that a blood vessel is included between a thin layer of glass (serving as a cover glass) and a thicker one serving as a slide. After a time the epidermis adheres to the edges of the chamber and blood vessels, nerves and other tissues grow into it where they can be studied under oil immersion objectives. This technique was first reported by Sandison (J. C., Anat. Rec., 1924, **28,** 281) working under Dr. E. R. Clark at the University of Pennsylvania. It has since been very greatly improved (Clark, E. R., et al., Anat. Rec., 1930, **47,** 187–211 and Abell, R. G., and Clark, E. R., Anat. Rec., 1932, **53,** 121–140) by the introduction of "round table" and "moat" chambers.

To place a window in the wall of the skull and to observe what is going on within has been done with more or less success on several occasions. The technique devised by Forbes (H. S., Arch. Neurol. and Psych., 1928, **19,** 75) permits direct observation at low magnification of the blood vessels over the cerebral convolutions with so little injury that their behaviour in various experimental conditions can be investigated (see also Clark, E. R., and Wentsler, N. E., Proc. Assoc. Res. Nerv. and Ment. Dis., 1937, **18,** 218–228). Through a window in the thoracic wall Wearn and his associates (Wearn, J. T. et al., Am. J. Physiol., 1934, **109,** 236–256) have similarly studied the pulmonary arterioles and capillaries. They employed a fused quartz cone to conduct light to the tissue. For collection of alveolar fluid see Terry, R. J., Anat. Rec., 1926, **32,** 223–304; 1936, **64,** 75.

Other investigators have availed themselves of the natural window, the cornea, through which what goes on immediately within it in the anterior chamber of the eye can be observed. Several tissues have been successfully transplanted into this chamber. Perhaps the most dramatic is the behavior of transplanted uterine mucosa in the rhesus monkey. In it the menstrual changes can be seen in detail and the influence of hormones noted (Markee, J. E., Contrib. to Embryol., Carnegie Inst. of Washington, 1940, **28,** 219–308). For some kinds of work the fact that the tissue fluid (aqueous humor) in this chamber differs from others in the same animal by the absence of certain species specific growth inhibiting factors is a priceless asset. Thus Greene (H. S. N., Science, 1938, **88,** 357–358) was able to grow pieces of human cancers, which ordinarily quickly die in other species, in the anterior chambers of the eyes of some mammals. The existence of a barrier protecting this fluid against the entry of antibodies from blood plasma and thus making possible the growth of tumor transplants, while all other tissues are resistant to their growth, has recently been emphasized (Saphir, O., Appel, M. and Strauss, H. A., Cancer Res., 1941, **1,** 545–547).

In order to view the less accessible living tissues, techniques have been devised that include opening the body and partly withdrawing the organ so that it can be placed on the stage of a microscope but with circulation and nerve supply intact and adequate regulation of temperature and humidity. Particularly fruitful has been the direct observation through oil immersion objectives of secretion by acinous cells of the **Pancreas** by Covell (W. P., Anat. Rec., 1928, **40,** 213–223) and of islet cells by O'Leary, (J. L., Anat. Rec., 1930, **45,** 27–58). Thus the influence of drugs on the secretory process can now be followed in minute detail.

Knisely (M. H., Anat. Rec., 1936, **64,** 499–523; **65,** 23–50) has perfected a technique for the study of the living **Spleen** at somewhat lower magnification. The essential features are slight displacement of the spleen so that it can be transilluminated by light delivered through a quartz rod. This allows for the first time direct examination of the behavior of the venous sinuses. Undoubtedly the **Quartz Rod** technique will be of great service in providing light for similar examination of other organs.

### 3. To Mark Tissues in Vivo by **Vital Staining** or in Other Ways for Later Examination

In this connection we at once think of the vital stains, trypan blue, carmine, India ink (carbon) and hundreds of others, which, when injected into the body, are phagocytosed by the reticulo-endothelial cells (or macrophages). Pieces of tissue can then be excised and the accumulations of stains can be studied within the still living cells, that is supravitally, for unless cultured the cells are slowly dying. But, if desired, the tissues can be fixed and the results observed at leisure in sections.

Another example of *in vivo* marking is the deposition of **Prussian Blue.** Thus a slightly hypertonic solution (potassium ferrocyanide 0.5 gm., iron ammonium citrate, 0.5 gm. and aq. dest. 50 cc.) injected into the subarachnoid space of the spinal cord is useful in the localization of the pathways of drainage of cerebrospinal fluid, because of the marking secured when the tissues are fixed in 40% formalin plus 1% concentrated hydrochloric acid by the deposition of Prussian blue (Weed, L. H., J. Med. Res., 1914, **26,** 21–117).

### 4. To Mark Still Living Tissues by **Supravital Staining** and to Study Them Later

This is done by **Perfusion** of the blood vessels with dyes some of which are too toxic for intravenous injection in living animals. The dyes thus supplied to the tissues in a supravital way are often picked up by certain cells with truly remarkable specificity.

The outstanding methods in this group have been devised by Bensley (R. R., Am. J. Anat., 1911, **12,** 297–388) for histological analysis of the epithelial components of the pancreas and stomach. Dilute solutions of the dyes in physio-

logical saline are injected into the thoracic aorta of an animal killed by bleeding. Pieces of pancreas and gastric mucous membrane are then removed and examined fresh. Neutral red picks out the **Islets of Langerhans** of the pancreas, pyronin the duct system of the pancreas, naphthol blue the parietal cells of the **Stomach** and so on. In the same way **Nerve Fibers** can be marked for subsequent study by vascular perfusion with methylene blue and degenerating nerve fibers in poliomyelitis (and presumably in other conditions) can be sharply differentiated from uninjured ones by the fact that they take up neutral red (Covell, W. P. and O'Leary, J. L., Arch. Neurol. & Psych., 1932, **27**, 518–524). It has long been known that the best way to mark renal glomeruli is to perfuse in the same fashion with a dilute solution of janus blue. The glomeruli stand out clearly in the fresh kidney by their deep blue color in a red background (Cowdry, E. V., Contrib. to Embryol., Carnegie Institution of Washington, 1918, No. 25, 39–160). A similar selective staining in less brilliant colors is obtainable with janus green. Relatively permanent preparations can be made of some of these specimens.

The same dyes, and many others, can also be applied in dilute solutions to cells freshly removed from the body and which are still living. Such methods have become very popular in hematology. However, the cells thus colored live only for a limited time and it is important to cut short the observations before they are vitiated by approaching death.

### 5. To Employ Tissue Cultures

The method of tissue culture has two great advantages over all other techniques for the study of living cells. It offers unrivalled opportunities for experimentally changing their fluid environments and for their direct examination at high magnification over long periods of time thus permitting analysis of morphological changes by moving pictures. This is by far the best approach for direct studies of cellular motility, phagocytosis and several other physiological phenomena.

The limitation of the method is the obvious one that the fluid environments are artificial and must be changed at intervals to keep the strains of cells alive. Consequently tissue cultures are unsatisfactory for the investigation of intercellular materials, like fibers, hyaline deposits and so on. Moreover the cells cannot properly organize to form tissues and organs as they do *in vivo* since they are isolated from normal influences by other tissues of the body. But they make the effort. Methods have recently been advocated for the culture of organized tissues, bones, teeth, etc. (Fell, H. B., J. Roy. Micr. Soc., 1940, **60**, 95–112).

In selecting the technique of tissue culture for the solution of any problem it is well to remember that considerable equipment and several years training are required to realize its full usefulness. For this reason valuable time will be saved by learning the technique from an expert. See Parker (R. C., Methods of Tissue Culture, New York, Hoeber, 1938, 292 pp.).

### 6. To Make **Microdissections** and **Microinjections** of Living Tissues

In some problems it is necessary to obtain accurate data on the character of the connections between fibers, living cells and parts of cells and on the physical properties of cellular and nuclear membranes and of cytoplasm and nucleoplasm. Through the introduction of suitable apparatus, surgical methods can now be employed with great accuracy under oil immersion objectives. Some therefore call the technique "micrurgical." Living cells and nuclei can also be injected with various substances. This is of great importance because it is often the only way to secure their direct entrance. Long practice is necessary under the watchful eyes of an expert but the results are worth it.

### 7. To Employ Different Kinds of **Illumination**

Direct light is naturally chosen for routine microscopic examinations and is the best for some special purposes. But it is often helpful to have the light come in from the sides at acute angles and to observe, in a dark field, the structures brilliantly illuminated by this light reflected from their surfaces. In this way many tiny objects, such as spirochetes, can be easily detected unstained, the mineral residues after **Microincineration** can be brightly illuminated, **Chylomicrons** can be distinctly seen, etc.

Visible light can also to advantage be **Polarized** when one wishes to detect doubly refractile, crystalline materials such as **Silicon** and certain **Lipids.** The newer **Polarization Optical** methods have opened a new field in the investigation of the molecular constitution of cell membranes but they require considerable training and knowledge of physics.

**Ultraviolet Light** is occasionally resorted to for the making of photomicrographs of some tissue components and it is essential in the study of fluorescence. By employing **Fluorescence Microscopy** the porphyrins, tubercle bacilli, uranium, vitamin A and other materials can be identified and their distribution in the tissues traced with great accuracy.

The RCA **Electron Microscope** is now available but at considerable cost. It gives great magnifications and undoubtedly reveals structural details which can be seen in no other way. The first step is for those interested to visit a laboratory in which one is being operated by some qualified person and to discover whether its use is necessary for their particular problems.

The electron microscope, constructed by Dr. G. H. Scott and his associates at Washington University, is an analytical instrument of great promise at present employing relatively low magnifications to show calcium and magnesium without the inclusion of any other elements. Those who think that it may be of assistance to them should consult Dr. Scott who is now Professor of Anatomy at the Medical School of the University of Southern California in Los Angeles.

### 8. To Study Minerals by **Histospectrography** and **Microincineration**

Histospectrography is essentially a survey technique because in the spectra obtained the lines of many substances are revealed any one of which it may be

important to further study by other techniques. The amounts of tissue sparked are small, but it is not possible to obtain the spectra of individual cells or of several cells of the same sort in their natural locations. Some cells can, however, be collected by centrifugation, as will be mentioned later, in adequate volumes for spectrographic analysis.

Microincineration, on the other hand, does demonstrate the minerals that are not volatilized at the temperature required (Ca, Mg, Fe, Si, Na, K) in very nearly the same if not quite the positions that they occupied in the living state. It is however not feasible as yet to determine the proportions of Ca and Mg or of Na and K in any particular mineral residue. This method is nevertheless one of wide usefulness. The incinerators are of moderate cost and the dark field apparatus used to examine the sections is at hand in most laboratories.

### 9. To Employ Various **Microchemical Reactions**

An increasing number of these are of proved value and they yield information which very advantageously links histology and biochemistry. Accuracy in the microscopic localization of elements and chemical substances is of course much to be desired. In making a selection see the list of microchemical reactions and consult a chapter on histochemical changes by Lowry, O. H. and Hastings, A. B. in Cowdry's Problems of Ageing, Baltimore: Williams & Wilkins Co., 1942, 728–755.

### 10. To Collect by Centrifugation Tissue Components in Sufficient Volume for Direct Chemical Analysis

Until recently the only cells which could be collected by themselves in sufficient quantities were sperms and erythrocytes. Now many others, suitably separated and washed, even their individual parts, nuclei, mitochondria and so forth, can be amassed by centrifugation and subjected to standard methods of analysis. Much has already been accomplished and more will assuredly follow. The ultracentrifuges which have been developed in the last few years are of unique service in diverse lines of research including the collection of still other tissue components, the determination of relative intracellular **Viscosity,** the concentration of proteins and viruses, the establishment of particle size and so forth. See **Centrifugation.**

### 11. To Investigate Physiological Properties

The classical physiologist studies function *en masse.* It is for the histologist, who is also a physiologist, in cooperation to investigate the functions of individual cells and tissues. In choosing techniques he is handicapped because he must use micromethods. Some physiological properties, like phagocytosis, motility and nutritional requirements, can, as already stated, best be examined in tissue cultures. Hydrogen ion concentration can be determined by suitable **Indicators** likewise oxidation-reduction potential. The motion picture technique, using fairly high magnification, is of value, because the various morphological changes

can be timed and individual responses of cells and tissues repeatedly examined for thorough analysis of stages and sequences. The sites of production of **Enzymes** (see list) can be localized more accurately than ever before and the factors that stimulate and inhibit their formation can consequently be more sharply analysed.

The great physiological problem of the replacement of tissues to compensate for wear and tear is being clarified by the introduction of new techniques. The investigator can if he wishes estimate the production of new cells by counting mitoses conveniently arrested in the metaphase for his inspection by the action of **Colchicine.** He can also form a fairly accurate idea of chemical replacement by **Vital Staining** of bony matrix laid down within a given time and in many tissues by the accumulation of radioactive potassium, iron and other elements. The field is wide open. All morphological methods give physiological information and *vice versa.*

### 12. To Use Methods of Maceration and Corrosion

Since the body is structurally so very complex it is often illuminating to view parts of it in their normal shape and size but unobscured by all the neighboring components. There are several ways by which this can be accomplished.

The first method of **Reconstruction** from serial sections is well known. Briefly stated the particular tissue, organ or system is outlined, as it appears in section after section, at the desired magnification on sheets of material of uniform and carefully selected thickness. The outlined areas are then cut out and when superimposed they constitute a reconstruction of the original structure. This technique is tedious but it may reveal topographical relations that can be discovered by no other means.

The second kind of technique is to make casts of vascular, respiratory and other lumina. Woods' metal, formerly used for this purpose, has now been almost displaced by **Celloidin** and other substances. The surrounding tissue is freed from the cast by digestion in concentrated hydrochloric acid and gentle brushing away in a stream of water. Very beautiful **Corrosion** preparations of the lungs and kidneys have been obtained by this method which should be more widely employed.

The third is by **Maceration** to soak the organs, without previous preparation, in fluids that either digest away the tissues which it is desired to eliminate or loosen their connections with those under investigation, which, latter, can then be individually examined. Techniques of this sort are the only available means for the isolation of individual seminiferous and renal tubules. Oliver's researches on the kidney illustrate the value of reconstruction and maceration in pathology. Only three other examples will be submitted. **Thyroid follicles** can be isolated by maceration (Jackson, J. L., Anat. Rec., 1931, 48, 219–239). Their study as individuals provides data as to size and shape only obtainable otherwise by the tedious examination of serial sections. The **Epidermis** is so tightly bound to the underlying dermis that separation is extremely difficult; but, after treatment

of skin with dilute acetic acid, the attachment is loosened and the epidermis can readily be removed as a complete sheet of tissue which can be stained, made transparent and examined as a whole mount. Opportunities are thus afforded for the detection of regional differences which might not be located even by painstaking study of sections and the making of mitotic counts is greatly facilitated. By macerating in the same fashion the nasal mucous membrane covering the septum can also be removed for study. Perhaps still other epithelial sheets can be similarly isolated. However such sheets are of little value for chemical analysis because of the action of the acetic acid. Fortunately it has been found that the epidermis may also be quickly loosened by simply heating the skin to 50°C. when it can be peeled off like the covering of a scalded tomato (Baumberger, J. P., Suntzeff, V. and Cowdry, E. V., J. Nat. Cancer Inst., 1942, **2**, 413–423).

There is still another alternative. Instead of simply omitting the unwanted material by reconstructing only the structures chosen for demonstration, or of removing the material by corrosion or maceration, it can be left in and rendered transparent so that it does not obstruct the view. After marking the particular structures by vital dyes or other means the whole tissue is cleared by the method of **Spalteholz** or **Schultze.** These techniques give admirable results in the study of **Cartilaginous Skeletons, Ossification** centers, **Blood Vessels** and so on almost without end.

### 13. To Employ the More Routine Method of **Fixation** and **Staining**

Here there is wide latitude of choice. For some purposes thin **Smears** are just fixed and stained without resort to sectioning. In the case of the denser tissues which must be cut in sections one first has to decide which of many **Fixatives** is likely to give the best results. Then, whether fixation is to be by immersion or injection has to be determined.

The purpose of fixation by vascular injection is to bring the fixative into close contact with the tissues as they exist in the freshly killed animal without subjecting them to mechanical trauma or disturbing their topographic relations one to another. In choosing this procedure it is well to remember: (1) That it is usually necessary first to wash out most of the blood by perfusion with physiological salt solution for otherwise the fixative often clogs the vessels. This washing unfortunately also facilitates chemical change. (2) That, even when it is not done, the concentration of the fixative about the cells is gradually increased and at different rates, rapidly in highly vascularized tissues (kidney, liver, etc.) and very slowly in avascular ones (epidermis, cornea and cartilage). The time for chemical change before fixation is therefore variable depending upon the tissue. (3) That the pressure may bring about an unnatural swelling of the tissues so located that they can enlarge, especially the abdominal organs as compared with brain and bone marrow which are confined within rigid walls.

Fixation by immersion is the usual and easiest method. If small pieces or thin slices are used the preservation is quicker and more uniform than by vascu-

lar injection. The cells are suddenly killed while active. The factor of slow death at uneven rates, present in supravital examinations, does not have to be reckoned with; but many precautions are required. Under **Fixation** is given a general account of the procedure. Under the several organs, **Lungs, Small Intestine, Skin,** etc., some special suggestions are provided. There are many fixatives to choose from. For routine purposes **Zenker's Fluid** as originally described or in one of its numerous modifications is suggested. **Bouin's** is also a very popular fixative especially among dermatologists. **Formalin** is an excellent one. It is good practice to set aside some tissue in formalin for examination as may be needed later. Both formalin and alcohol are the most useful fixatives preliminary to microchemical determinations. When preparations must be made very quickly, **Alcohol Formalin** and **Carnoy's Fluid** are suggested (see also **Frozen Sections**). For microincineration, formalin-alcohol is ordinarily employed; but the **Altmann-Gersh** method of freezing and drying, by which contact with fixatives is altogether dispensed with, is much less open to criticism. Osmic acid containing fixatives penetrate poorly and are therefore only useful for very small pieces of tissue. Regaud's fluid with subsequent mordanting in bichromate is the best for mitochondria. Heat fixation is useful for blood cells. Fixation in various vapors is called for in special cases. See **Fixatives.**

After fixation some **Washing** of the tissue in water is necessary unless it has been fixed in alcohol, Carnoy or similar mixtures. The next step is **Dehydration** and a choice must be made between slow and rapid methods. Sometimes a substitute for alcohol is indicated. If **Imbedding** is to be in celloidin **Clearing** in a xylol-like fluid is omitted and heating is unnecessary. There are many ways of clearing preliminary to paraffin imbedding. In **Sectioning** the thickness depends upon the purpose in view. Thick sections may be as necessary as thin ones and serial sections are often required. In the **Mounting** of sections on slides the use of water must occasionally be avoided. Numerous techniques are applicable to the sections and are given individually later either under the heading of the substance or structure to be demonstrated or under the name of the technique or its introducer. For choice see **Staining.**

### 14. To Make Quantitative Measurements of Selected Tissues

It not infrequently happens that data are wanted that can only be secured by accurate measurements. The counting of **Nerve Fibers, Leucocytes** and so on is not difficult and requires no higher mathematics. To measure length a micrometer ocular or ruled disk standardized by reference to a micrometer slide is required. To estimate **Volumes** is feasible but more of a job. Even the total **Mitochondrial Surface** in a cell can be measured fairly accurately. Estimations of mitotic rate depend upon counts of dividing and non-dividing nuclei (see **Mitosis**). The thickness of **Cell Membranes** can be determined with fair accuracy but it is a task for biophysicists. The same can be said for measurements of **Radioactive** substances in the several tissues, though the interpretation of radio-autographs is largely histological. Often measurements on models made

by **Reconstruction** are of value. The method of **Centrifugation** gives data on relative intracellular specific gravities and viscosities and on particle sizes. Another technique which is basic in so far that it is applicable in many situations is the **Photoelectric** measurement of color in microchemical reactions and of reflected light in the dark field examination of sections subjected to microincineration. To see the color is something, to measure it quantitatively is much more. Indeed the value of measurement is implicit in all microscopical work. Quantitative histology is almost a science in itself. Policard, at Lyon, is a leader and it is profitable frequently to consult his publication, the Bulletin d'Histologie Appliquè.

### 15. To Detect Deviations from Normal

The **Normality** of a tissue or organ is often in doubt. There is no single technique capable of yielding an unqualified answer. Since some properties may be normal while others are abnormal (pathological) we need first to be told the property under consideration. If it is, for instance, the amount of contained pigment, this can be said to be normal when it is the amount usually present in a particular tissue under the same conditions. By the word "usually" is intended in the majority of cases, that is in 51 per cent or in any higher percentage. The phrase "same conditions" means that the conditions likely to influence the amount of pigment are so nearly alike as to be not responsible for any difference observed between the property of the tissue where normality is in question and that of others of the same kind. Thus, we could say with reasonable assurance that the amount of pigment is normal if it is that usually demonstrated by the same technique in tissues of the same kind of animals of the same species, sex and age living under the same conditions. Judgment is necessary in specification of possibly modifying conditions which will depend to some extent on the property under consideration and on the number of observations necessary to establish the percentage within the limits of probability. It would not do to compare the amount of pigment in the specimen, the normality of which is in question, with that in too few others. This is the statistical definition of normality which is not universally accepted but which is useful and easily understood.

Only a few samples of the various kinds of technique have been mentioned in this survey as a kind of menu from which to make a selection or to obtain clues to other methods that may fit the case. Many of them are very ingenious and were only discovered after wisely conceived attempts to overcome practical difficulties. This overcoming of obstacles is a pleasant experience. It calls for actual work and experiment and appeals to many of our best minds. The techniques may be regarded as keys by which scientific treasure can be unlocked. Unused they are worthless.

## ORGANIZATION OF LABORATORY

Much lost motion is eliminated and efficiency is gained if careful attention is given to organization of the laboratory for the purposes intended. Wherever microscopic work is done in the laboratories of high schools, colleges, universities, medical, dental, agricultural and veterinary schools, hospitals, research institutes and biological and medical supply organizations there are certain common needs.

The first of these is to convince the Principal, Dean or Director that the sooner the archaic idea that the requirements may be reduced to any old microscope, a sharp knife, plenty of alcohol and time for meditation is abandoned the better. The best available apparatus is none too good and it costs money to buy and to operate. To correlate histological and biochemical techniques so that they will accurately reveal physiological and pathological changes is not a trivial task.

Few experiences are more wearing on the nervous system than to have to use a worn out microscope with faulty focussing apparatus and light which is never strong enough, both in the absence of a mechanical stage. Modern binocular microscopes with apochromatic lenses, fitted also for dark field examinations, are needed plus the best possible artificial illumination.

The rotatory and freezing microtomes must be in good working order. Each person who uses one of them regularly should have a microtome knife for the sharpness of which he alone is responsible. Two incubators are needed, one for paraffin imbedding and the other for drying paraffin sections. An ice box is required to keep fresh tissues, special chemicals, etc. Coarse and fine balances are essential for weighing. Of prime importance are accommodations for experimental animals which will always bear inspection including cages that can be easily sterilized. For special studies a fluorescence microscope, a microincinerator and other equipment are obviously necessary.

Difficulties will often evaporate if the pH of solutions is controlled. Consequently such determinations must be occasionally made and a glass electrode should be available. A recent textbook of biological chemistry ought to occupy a prominent place on the shelf. Because some technical procedures may be held up for lack of an unusual chemical, copies of Merck's Index and of Eastman's Organic Chemicals are helpful. In case of special difficulty consult the National Registry of Rare Chemicals, (Dr. Martin H. Heeren, Director), Armour Research Foundation, 33rd, Federal and Dearborn Streets, Chicago. For histochemistry see Lison, L., Histochimie Animale. Paris, Gauthier-Villars, 1936, 320 pp. A book on dyes, like that of Conn, is frequently required. The standard list of stains, to which Conn and others make regular reference, is the Colour Index of the Society of Dyers and Colourists (Rowe, F. M., Colour Index, 1924, Bradford). Some nearby library might be induced to purchase it.

Work in any such laboratory should be constructive. By the routine collec-

tion of data on all tissues examined and the systematic filing of permanent preparations, paraffin and celloidin blocks as well as tissues fixed in formalin, a mass of material of very real value will accumulate through the years much of which can be used again and again. This is a measure of economy as well as a time saver. It is important also to protect the technician from undue demands. Members of the staff, visiting physicians and surgeons and colleagues in other departments not infrequently ask for many more preparations than they need, because they are occasional visitors and fail to realize what a lot of work is involved. The principle should be—a few preparations of the best, meticulously studied, with material set aside so that others can be made as needed.

In streamlining the laboratory the first essential is orderliness and neatness. It is possible for some geniuses to do outstanding research amid confusion worse confounded but ordinary mortals cannot. Depreciation in quality inevitably follows carelessness. Adequate provision for the regular and systematic cleaning of glass ware is one of the essentials. With increase in age a laboratory tends to become fixed in its ways. Techniques of proven worth are employed to the exclusion of others. New blood in the form of new methods is required to keep any laboratory a little ahead of the game.

## STANDARDIZATION OF STAINS

In the use of stains one encounters a multitude of names, many of which are synonyms, and it is difficult to be sure of their meaning. Two comprehensive dye indexes have been published. One, "Schultz' Farbstofftabellen", is now in its 7th edition (1928 to 1934) but confusion is created by the fact that the index numbers of the dyes given in it do not correspond to those in the earlier editions. The other, the "Colour Index of the Society of Dyers and Colourists", was edited by F. M. Rowe and published in 1924. It was followed in 1928 by a supplement, but there has been no second edition. This Colour Index gives (1) the commercial name, or much more frequently names for there are so many synonyms; (2) the formula, (3) the preparation, (4) the discoverer and (5) the properties of a vast assemblage of dyes. It is the standard of reference in the United States and other English-speaking countries. When one wishes to be specific it is customary to list after the dye used its colour index number, for example vital red, C. I. No. 456.

Much aid is given to investigators by the Biological Stain Commission and particularly by its distinguished Chairman, Dr. H. J. Conn. This commission is concerned with the inspection and standardization of stains, not with their manufacture as is sometimes supposed. It was found in 1920, while the post-war embargo on dyes was still in effect, that American scientists were being supplied with dyes from three or four different stain companies and that their products were not sufficiently uniform to be reliable. Accordingly, through the cooperation of the National Research Council and of several national scientific societies, the Commission on Standardization of Biological Stains was established. The Commission is now an independent organization but includes in its membership representatives of eight societies with which it cooperates. The work of the Commission is two-fold. First, by cooperation of biologists and chemists it gathers information concerning the nature of dyes as related to their use in microscopic technique; secondly, by working with the manufacturers and dealers it endeavors to see that the supply of available stains in America is of the highest possible quality as judged by their performance in actual laboratory use. The first of these purposes has inspired a useful book on "Biological Stains" by Conn, now in its fourth (1940) edition, and at the same time has led to the publication by the Commission of a quarterly, "Stain Technology." The second object is being brought about by the certifying of stains.

The certification plan has been adopted because of the great difficulty of drawing up any chemical or physical standards to determine which stains are satisfactory and which are not. If such standards were formulated, it would be possible to prepare specifications with which manufacturers of stains would be expected to comply. In the early work of the Stain Commission an attempt was made to draw up such specifications and they were published, in provisional

form, for a few stains in the first edition of "Biological Stains." Full specifications are given in the fourth edition and in the National Formulary.

Instead of drawing up specifications, therefore, the Stain Commission instructs the manufacturers of stains to submit samples to it of every batch manufactured of any of the stains that are on the certification basis. The Commission submits these samples to certain definite tests which have now been formulated and published (See Conn, pp. 246–276). The methods in question include chemical, spectrophotometric, and biological tests, and only those dyes are certified which are satisfactory in all these tests. Such dyes the manufacturers are allowed to sell with a special label on the package indicating approval by the Stain Commission.

The certification label on any bottle of stain means, therefore, that: (1) a sample of the batch bearing the label has been submitted to the Commission for testing and a portion of the sample is permanently on file in the chairman's office; (2) the sample proves true to type, as judged by spectrophotometric tests; (3) its dye content is up to specification and is correctly indicated on the label; (4) it has been tested by experts in the procedures named on the label and has been found satisfactory by them; and lastly, (5) no other batch can be sold under the same certification number except by such a flagrant breach of confidence on the part of the company as to risk losing the good will of the Commission. At present (1942) the following stains have been placed on the certified list. In descriptions of their use the names should be followed by C.C., indicating that the products were Commission Certified, for instance, alizarin red S (C.C.).

Alizarin red S
Anilin blue, water soluble
Auramine O
Azocarmine G
Azure A
Bismarck brown Y
Brilliant cresyl blue
Brilliant green
Carmine
Chlorazol black E
Congo red
Cresyl violet
Crystal violet
Eosin, bluish
Eosin, yellowish
Erythrosin B
Ethyl eosin
Fast green FCF
Fuchsin, acid
Fuchsin, basic
Giemsa stain
Hematoxylin
Indigo carmine
Janus green B
Jenner's stain
Light green, S.F., yellowish
Malachite green
Martius yellow
Methyl green
Methyl orange
Methyl violet 2B
Methylene blue chloride
Methylene violet
Neutral red
Nigrosin
Nile blue A
Orange G
Orange II
Orcein
Phloxine
Pyronin
Resazurin
Rose bengal
Safranin O
Sudan III
Sudan IV
Tetrachrome stain (MacNeal)
Thionin
Toluidine blue O
Wright's stain

Eight companies in the United States are now submitting their stains to the Commission for certification before putting them on the market. It must be realized, however, that no one of these concerns necessarily *manufactures* all the stains which it thus submits; but in the case of any stain which is manufactured elsewhere, the company takes responsibility for its performance as a biological stain, on the basis of tests made to show its adequacy, and in many instances carries out a certain degree of purification or other processing before putting the stain on the market. One of these companies puts on the market every stain now on the certification list. Two other companies submit samples of over half the stains thus listed, while the other companies merely request certification of one or two dyes in which they specialize. No dyes have yet been certified by the Stain Commission submitted by any foreign concern. The reason for this is because of the difficulty in handling the certification of stains on the batch basis with a concern that is located at a distance, doing business in this country only through agents who are not in direct touch with the actual manufacturers of the dyes. Cooperation among the Americas is increasing (Conn, H. J., Stain Techn., 1942, **17**, 5–6).

In several recent editions of the National Formulary, published by the American Pharmaceutical Association, a section has been included in which formulae of staining solutions are given. In the past there has been no agreement between these formulae and the ones recommended by the Stain Commission. Beginning in 1937, however, it was decided that the National Formulary Committee and the Biological Stain Commission should cooperate in this matter. Accordingly, the chairman of the latter was made a member of the former and a member of the National Formulary was put on the Executive Committee of the Commission. This interlocking membership is assurance that the work of preparing staining formulae for the next edition of the National Formulary is being carried on in close cooperation with the Stain Commission. This cooperation has already resulted in two important steps:

1. Specifications of the most important stains now on the certification basis have been drawn up for the National Formulary (1942). These specifications are partly chemical and spectrophotometric, but also contain detailed statements as to how the stains should be tested as to their behavior for biological purposes and state the results to be expected from these tests. In every case these specifications have been made to harmonize with the tests as actually performed by the Stain Commission.

2. The formulae given in the National Formulary, in "Biological Stains" and in the "Manual of Methods for the Pure Culture of Bacteria," published by the Society of American Bacteriologists, have been compared and critically studied with the object of making them identical in all three.

## ABBREVIATIONS

1 $\mu$ (Greek letter for micron) = 1/1000th part of a millimeter (mm.) = 0.001 mm. = $10^{-3}$ mm. = 10,000 Å = approximately 1/25,000th of an inch.
1 m$\mu$ (millimicron) = 1/1000th part of a micron = 1/1,000,000th part of a mm. = $10^{-6}$ mm. = 0.001 $\mu$ = 10 Å.
1 Å (Angstrom unit) = 0.1 m$\mu$ = 0.0001 $\mu$ = $10^{-7}$ mm.
1 $\mu\mu$ (micromicron) = 1/1,000,000th part of a micron = 1/1,000,000,000th part of a mm. = $10^{-9}$ mm. = 0.000,001 $\mu$ = $10^{-2}$ Å.
1 Kg. = approximately 2.2 lbs.
1 gm. = $10^{-3}$ Kg., 0.001 K., 1000 mgm., 1,000,000 $\mu$g.
1 mgm. = $10^{-6}$ Kg., $10^{-3}$ gm., 1000 $\mu$g.
1 $\mu$g. = 1$\gamma$ = $10^{-9}$ Kg., $10^{-6}$ gm., $10^{-3}$ mgm.
*N* NaCl is normal solution of sodium chloride, see **Normal Solution.**
*M* HCl is molecular solution of hydrochloric acid, see **Molecular Solution.**
*M* = mole.
mM = millimole.
ME = milligram equivalent.
1 ml (milliliter) = 1/1,000th part of a liter = 1 cc. (approx.).
CI 76 means that the number of a dye is 76 in the Colour Index of the Society of Dyers and Colourists.
CC. given after a dye signifies that it has been certified by the Biological Stain Commission.

The following publications are simply referred to by author, or senior author, or editor's name and page number (cf. Conn, p. 26).


Bensley, R. R. and S. H., Handbook of Histological and Cytological Technique, Univ. Chicago Press, 1938, 167 pp.
Bourne, G., Cytology and Cellular Physiology, Oxford: Clarendon Press, 1942, 296 pp.
Conn, H. J., Biological Stains, Geneva, N. Y.: Biotech Publications, 1940, 308 pp.
Cowdry, E. V., Textbook of Histology, Philadelphia: Lea & Febiger, 1938, 600 pp.
Downey, H., Handbook of Hematology, New York: Hoeber, 1938, 3136 pp.
Lison, L., Histochemie Animale, Paris: Gauthier-Villars, 1936, 320 pp.
Mallory, F. B., Pathological Technique, Philadelphia: Saunders, 1938, 434 pp.
McClung, C. A., Microscopical Technique, New York: Hoeber, 1938, 698 pp.
Stitt, E. R., Clough, P. W. and M. C., Practical Bacteriology, Haematology, and Animal Parasitology, Philadelphia: Blakiston, 1938, 961 pp.

**A-V Bundle,** see Todd, T. W., Cowdry's Special Cytology, 1932, 2, 1173–1210.

**Absorption** of water, see **Water Absorption** and fat absorption after previous coloration of fat with Sudan III or Sudan black (see **Vital Staining**).

**Absorption Spectra.** Methods are available for the determination of absorption spectra of cell structures. Casperson (T., J. Roy. Micr. Soc., 1940, **60**, 8–25) has described apparatus for absorption from intracellular objects larger than 1 micron such as Nissl bodies. This line of investigation is just developing and is likely to be productive of important results. See **Histospectroscopy.**

**Acacia,** properties as a macromolecule (Hueper, W. C., Arch. Path., 1942, **33**, 267–290).

**Acanthocephala,** see **Parasites.**

**Acarina,** see **Parasites, Ticks.**

**Acetic Acid** (L. acetum, vinegar). Widely used as a component of fixatives. The undiluted solution is often termed "glacial acetic acid." This contains 99.5% $CH_3COOH$. Causes a distinctive swelling of fresh collagenic fibers. Employed in dilute solution to destroy red blood cells so that whites can be examined. In 1% solution separates epidermis from dermis. See **Epidermis.**

**Acetic-Osmic-Bichromate** fixative of Bensley. 2% osmic acid, 2 cc.; 2.5% aq. potassium bichromate, 8 cc.; glacial acetic acid, 1 drop. Excellent for mitochondria but very small pieces of tissue must be used because the fluid penetrates poorly. The best stain is **Anilin-Fuchsin Methyl Green,** see also **Copper Chrome Hematoxylin.**

**Aceto-Carmine** (Schneider's). Add 10 gms. carmine to 100 cc. 45% aq. glacial acetic acid. Dissolve with heat and bring up to boiling. Cool, filter, and store as stock solution.

**Acid Alcohol** is used for the differentiation, or decolorization, of certain stains. It is usually made by adding 1 cc. hydrochloric acid to 99 cc. 70% ethyl alcohol. It is also employed for cleaning cover glasses.

**Acid Alizarin Blue** (1) G.R. (CI, 1048). An acid anthraquinone dye called for in **Buzaglo's Method** which the author proposes as substitute for Van Gieson.

(2) B.B. (CI, 1063) likewise an acid anthraquinone dye little used, if at all.

**Acid Bordeaux,** see **Bordeaux Red.**

**Acid Congo R,** see **Vital Red.**

**Acid Dyes,** see **Staining.**

**Acid Fast Bacilli.** Of these the organisms of tuberculosis and leprosy are the most important.

1. In smears apply **Carbol Fuchsin** gently heat 3–5 min. or stain room temperature 15 min.; decolorize 95% ethyl alcohol containing 3% of conc. hydrochloric acid until only slight pink color remains; wash in water; counterstain sat. aq. methylene blue or **Loeffler's Alkaline Methylene Blue;** wash and dry.

2. In sections the organisms can be stained *red* in paraffin sections after almost any fixation (formalin-Zenker preferred). First color with Harris hematoxylin. Wash in water and perhaps decolorize a little in **Acid Alcohol.** Wash again. Stain with warmed carbol fuchsin 1 hr. or more. Decolorize in acid alcohol. Wash carefully in water plus few drops ammonia. 95% alc., abs. alc., xylol, balsam. A critique of the methods has been published (Fite, G. L., Am. J. Path., 1938, **14**, 491–508). To color the organisms *blue*, fix 3–5 days or more in equal parts 10% formaldehyde and 95% alcohol. Stain sections in new fuchsin 0.5 gm.; phenol crystals, 5.0 gm.; alcohol methyl or ethyl, 10 cc. + aq. dest. to make 100 cc. at 60° C. over night, 12–24 hrs. or at room temperature 24–48 hrs. Longer for *M. leprae.* Freshly distilled aq. formaldehyde 5–30%, 5 min. 2% hydrochloric acid in 95% alcohol, 5 min. 1% aq. potassium permanganate 2–5 min. (until brown). 2% aq. oxalic acid, 1 min. Harris' hematoxylin 2 min. Stain in acid fuchsin, 0.1 gm.; picric acid, 0.5 gm.; aq. dest. to make 100 cc. Without washing, dehydrate in alcohol, clear in xylol and mount in balsam. Nuclei, brown; connective tissue fibers, red; muscle, yellow; acid fast bacilli, dark ultramarine blue. Good for photography (Fite, G. L., J. Lab. & Clin. Med. 1939, **25**, 743–744).

3. Mr. J. M. Albrecht employs the following method in our laboratory. Deparaffinize 5–6$\mu$ sections of 10% formalin or Regaud fixed tissues. Wipe off excess water around sections and cover with strip of filter paper. Flood filter paper with carbol fuchsin (Phenol crystals, 8 gm.; basic fuchsin, 4 gm.; 95% ethyl alcohol, 20 cc.; aq. dest., 100 cc.). Steam for 3 min. and then allow to stand for 30 min. adding more stain if necessary. The filter paper prevents deposition of ppt. of dye on sections. Flush off stain with aq. dest. Partly

differentiate in 1 cc. conc. hydrochloric acid in 100 cc. 70% alcohol, sections becoming deep pink. Wash in aq. dest. Stain **Harris' Hematoxylin** 10 min., wash in aq. dest. Complete differentiation of both fuchsin and hematoxylin in 50 cc. 70% alc. + 4–5 drops hydrochloric acid, sections becoming light pink. Wash in aq. dest. Neutralize in 6 drops conc. ammonia + 50 cc. aq. dest. Wash, dehydrate, clear and mount as usual.

See **Tubercle** and **Leprosy Bacilli, Fluorescence Microscopy,** also paper by Richards, O. W., Kline, C. K. and Leach, R. E., Am. Rev. Tuberc., 1941, **44,** 255–266.

**Acid Fuchsin** (CI, 692)—acid magenta, acid rubin, fuchsin S, SN, SS, ST or S III—Commission Certified. Since this is a sulfonated derivative of basic fuchsin, and, because there are 4 possible primary basic fuchsins, Conn (p. 118) points out that at least a dozen primary acid fuchsins are possible and samples are usually mixtures of several. Acid fuchsin is employed is so many ways that to enumerate them would be both futile and unnecessary. See **New Fuchsin.**

**Acid Green,** See **Light Green SF** yellowish.

**Acid Green O,** see **Naphthol Green B.**

**Acid Hemalum,** see **Hemalum.**

**Acid Magenta,** see **Acid Fuchsin.**

**Acid Orange II, Y** or **A,** see **Orange II.**

**Acid Phloxine GR,** see **Chromotrope 2R.**

**Acid Rubin,** see **Acid Fuchsin.**

**Acid Violet.** Several triphenyl methane dyes come under this heading. Conn (p. 132) says that the term "acid violet" is too indefinite for identification. This is unfortunate because dyes bearing this label have been used in several combinations as in Bensley's **Neutral Safranin** acid violet. Bailey, P., J. Med. Res., 1921, **42,** 349–381 and Maurer, S. and Lewis, D. D., J. Exp. Med., 1922, **36,** 141–156, working in Bensley's laboratory, used it for the pituitary. Acid violet is one of the stains employed by Weiss, E., J. Inf. Dis., 1928, **43,** 228–231 to stain flagella and spirochetes (J. Lab. & Clin. Med., 1928–29, **14,** 1191–1193).

**Acid Yellow,** see **Fast Yellow.**

**Acid Yellow R,** see **Metanil Yellow.**

**Acidity,** see **Hydrogen ion indicators.**

**Acidophilic,** see **Staining.**

**Acids,** see under first name, **Acetic Acid, Hydrochloric Acid,** etc.

**Acridine Dyes.** As the name suggests they are formed from acridine which is related to xanthene. Examples: acriflavine, neutral acriflavine and phosphine. Phosphine 3R is employed as a fluorochrome for lipids.

**Actinomyces.** Mallory's stain for actinomyces in sections (Mallory, p. 279). For the organisms, fixation in alcohol or in 10% formalin is preferable; but for the lesions, Zenker's fluid is better. Stain deparaffinized sections in **Alum Hematoxylin** 3–5 min. After washing in water stain in 2.5% aq. phloxine or in 5% aq. eosin in paraffin oven, 15 min. After again washing, stain in Stirling's or Ehrlich's aniline crystal violet (see **Anilin Crystal Violet**), 5–15 min. Wash in water and treat with **Gram's Iodine,** 1 min. Wash in water, blot and destain in aniline oil until no further color comes out. Rinse in xylol and mount in balsam. Branched forms, blue; clubs, pink to red.

**Adenosinase.** A method for analysis of adenosinase in lymphocytes and polymorphonuclear leucocytes (neutrophiles) is given by Barnes, J. M., Brit. J. Exp. Path., 1940, **21,** 264–275.

**Adrenal.** For routine purposes fix in **Zenker's Fluid** and stain paraffin sections with **Hematoxylin** and **Eosin.** There are many techniques for **Lipids.** The **Chromaffin Reaction** is often used for adrenalin but Cramer, W., J. Path. & Bact., 1937, **44,** 633, considers blackening with osmic acid vapor as more specific. Silver methods for vitamin C are difficult to apply but are apparently reliable. They are given under **Vitamins.** The **Schultz** cholesterol test gives excellent results. A selection may be made from several methods for **Reticular Fibers.** Corner, G. W., Contrib. to Embryol., Carnegie Inst., 1920, **9,** 87–93, employed for reticulum the Bielschowsky-Maresch silver method exactly as specified by Ferguson, J. S., Am. J. Anat., 1912, **12,** 277–296. The **Bodian** protargol method for nerve fibers has been adjusted to the adrenal by MacFarland, W. E., and Davenport, H. A., Stain Techn., 1941, **16,** 53–58, also **Cajal's** chloral hydrate method. If one contemplates ultracentrifugation and the demonstration of the Golgi apparatus consult Guyer, M. F., and Claus, P. E., Anat. Rec., 1939, **73,** 17–27.

**Adrenalin,** see **Chromaffin Reaction.**

**Agar,** as matrix for cutting plant material with freezing microtome (Evenden, W. and Schuster, C. E., Stain Techn., 1938, **13,** 145–146).

**Agonal Changes** are particularly difficult to avoid in villi of small intestine. They are evidenced by a ballooning of the epithelial cap most marked when absorption of ordinary food stuffs is active. The ballooning phenomenon can be produced in the living animal by ligating arteries of supply or by em-

ploying fixatives which induce forcible contraction of smooth muscle (Macklin, C. C. and M. T., Chapter on Intestinal Epithelium in Cowdry's Special Cytology, N. Y., Hoeber, 1932, **1**, 235).

**Albert's Stain** for **Diphtheria Bacilli**, which see.

**Albumen-Glycerin** for mounting paraffin sections. Egg white 50 cc., glycerin 50 cc., sodium salicylate 1 gm. Shake together and filter during several days. See also **Starch Paste** and **Masson's Gelatin Glue.**

**Alcohol.** Unless indicated to the contrary the word "alcohol" as employed in this book refers to the ethyl variety. Alone it is a good fixative preliminary to tests for **Amyloid, Copper, Fibrin, Glycogen, Gold, Hemofuscin, Hyaline, Iron, Lead, Palladium, Phosphatase, Potassium** and **Thallium**, which see. It is also employed in the demonstration of Nissl bodies by **Gallocyanin**, of mucus by **Mucicarmine**, of proteins by the **Romieu Reaction**, etc. In combination with other chemicals alcohol is also much used as a fixative, see **Alcohol Formalin, Carnoy's Fluid** and many others.

Alcohol of 70% is a good preservative and celloidin blocks can be stored in it. Absolute alcohol is supposed to contain not more than 1% by weight of water. It is considered to be 100 per cent. A very rough test for absolute alcohol is to mix with it a few drops of turpentine. If it becomes milky it contains too much water. To make a lower percent from a higher one by dilution take the number of cc. corresponding to the percentage required and add aq. dest. to make in cc. the percentage of the alcohol diluted. Thus to make 30% from 70% take 30 cc. of 70% and add aq. dest. to make 70 cc. Alcohol is the best dehydrating agent for tissues. It is sometimes not easy to purchase absolute alcohol so that it must be prepared. Take say 10 liters of 95% alcohol, add 400 gms. freshly ignited calcium oxide. Leave, with occasional shaking, 24 hrs. until most of the water is absorbed by the oxide. Pour off fluid (leaving oxide at bottom of container) and distill using appropriate precautions. Keep the "absolute" as nearly so as possible by using a tight glass stopper for the bottle, or in place of the stopper an absorption tube containing calcium chloride so that any water in entering air will be absorbed and will not reach the alcohol. See **Dehydration**, also **Amyl, n-Butyl, Tertiary Butyl, Isopropyl, n-Propyl** and **Polyvinyl Alcohols.**

**Alcohol-Formalin** is a fixative containing 9 parts of absolute alcohol and 1 part of formalin. Since it penetrates quickly and dehydration can be commenced in absolute alcohol immediately after fixation, skipping the lower grades of alcohol, permanent preparations can be be made within a few hours' time. For routine purposes 3–6 hrs. fixation will suffice but as a preliminary to **Microincineration** 24 hrs. is recommended. Alcohol-formalin is recommended for **Fibrin, Glycogen, Indigo-Carmine** stains and **Peroxidase.** It is employed with acetic acid in **Bodian's Method** for nerve fibers.

**Alizarin** (CI, 1027) a little used acid anthraquinone dye.

**Alizarin No. 6**, see **Purpurin.**

**Alizarin Blue RBN**, see **Gallocyanin.**

**Alizarin Carmine**, see **Alizarin Red S.**

**Alizarin Line Test** for new bone and vitamin D (Martin, G. J., J. Lab. & Clin. Med., 1940, **26**, 714–719). See **Line Test.**

**Alizarin Purpurin**, see **Purpurin.**

**Alizarin Red S** (CI, 1034)—alizarin red water soluble, alizarine carmine—Commission Certified. By far the most used of all the alizarin stains. An important ingredient in **Benda Method.** Much superior to **Madder** for the staining of bone and dentine laid down while it is in the circulation. Schour has employed it extensively. The technique is described in detail by him and his associates (J. Dent. Res., 1941, **20**, 411–418). He employed an Alizarin red S (CI, 1034) obtained from Coleman and Bell Co. The effective dose for rat, rabbit, guinea pig, cat, monkey and human infant is between 50–100 mg. per Kilo. conditioned by species, age and weight. For newborn white rats he recommends 0.2 cc. 2% Alizarin and for rats weighing 100–200 gms. ½–1 cc. given intraperitoneally. Colors are retained in specimens fixed in 10% neutral formalin or in 95% alc. As in the case of **Madder** staining of bone, tissues can be cleared and examined as whole preparations, or ground sections can be prepared for microscopic study. Decalcification spoils the color. See **Ossification** and **Line Test.**

**Alizarin Red Water Soluble**, see **Alizarin Red S.**

**Alkaline Methylene** blue, see **Loeffler's.**

**Alkaline Phosphatase**, see **Phosphatase** and **Kidney.**

**Alkalinity**, see **Hydrogen Ion Concentration.**

**Allen's Fluids** are modifications of **Bouin's** often containing urea. They are excellent for chromosomes.

**Alloxan Reaction.** 1% alcoholic solution of alloxan gives red color with $\alpha$ aminoacids. Romieu (M., Bull. d'Hist. appl., 1925, **2**, 185–191) employs a cold neutral solution. Giroud (A., Protoplasma, 1929, **7**, 72–98) uses heat but states that great

care is necessary in interpretation. See Lison, p. 129.

**Altmann's Fluid.** Equal parts of 5% aq. potassium bichromate and 2% aq. osmic acid. Employed in his method as well as for staining with **Copper Chrome Hematoxylin.** It gives good surface fixation but penetrates very badly.

**Altmann's Method** of anilin fuchsin and picric acid for mitochondria. Fix small pieces not more than 2 mm. in diameter 24 hrs. in **Altmann's Fluid.** Wash for 1 hr. dehydrate, clear imbed in paraffin and cut sections 4$\mu$. Pass down to water. Stain in anilin fuchsin (20% acid fuchsin in anilin water) 6 min. Blot with filter paper. Differentiate and counter stain by flooding the sections with 1 part sat. alc. picric acid and 2 parts aq. dest. Rinse rapidly in 95% alc., dehydrate in abs. alc., clear in xylol and mount in balsam. The mitochondria are stained crimson against a bright yellow background. Altmann's magnificent original plates should be examined (Altmann, R., Die Elementarorganismen und ihre Beziehungen zu den Zellen. Leipzig: Veit Co., 1894, 160 pp.). If these are not available see Meves, F., Arch. f. mikr. Anat., 1913, 82, (2), 215–260.

**Altmann-Gersh** freezing and drying technique (Gersh, I., Anat. Rec., 1932, **53,** 309–337). This method is of unique value since the tissues are frozen almost instantaneously in liquid air and are dehydrated *in vacuo* while still frozen. Fixation, alcoholic dehydration and clearing are omitted because the dried tissues can be immediately infiltrated with paraffin. Obviously it is the technique of choice for many microchemical studies and particularly for **Microincineration.** Significant improvements have been made by Scott and are described fully (McClung, pp. 647–652). The best method is to freeze small bits of tissue in isopentane which has been chilled with liquid nitrogen (Hoerr, N. L., Anat. Rec. 1936, **65,** 293–317; Simpson, W. L., ***Ibid.,*** 1941, **80,** 173–189). To avoid shift of inorganic salts Scott found dehydration *in vacuo* at −63°C. to be desirable. The apparatus required is known as a cryostat. It is not on sale but can be constructed on Scott's specifications. (Dr. Gordon H. Scott, Dept. of Anatomy, University of Southern California, Los Angeles).

**Alum.** The alums are double salts of sulphuric acid. Aluminum potassium sulphate, or potassium alum, unless otherwise stated is the one used in making up hematoxylin solutions. Aluminum ammonium sulphate, or ammonia alum, should not be used as a substitute unless called for. Ammono-ferric sulphate, or iron alum is used as a mordant and differentiator in the iron hematoxylin technique and for other purposes. The crystals are of a pale violet color. Their surfaces oxidize readily and become useless. The surface should be scraped off. Only the violet crystals are of any use.

**Alum-Carmine** (Grenacher). Boil 1–5% aq. ammonia alum with 0.5–1% powdered carmine. Cool and filter. Does not penetrate very well and hence is not suitable for staining large objects in bulk. But it is useful and does not overstain (Lee, p. 140).

**Alum Hematoxylin.** Many hematoxylin solutions contain alum, see **Delafield's, Ehrlich's, Harris', Mayer's.**

**Aluminium Chloride Carmine** (Mayer). Dissolve 1 gm. carminic acid and 3 gm. aluminium chloride in 200 cc. aq. dest. Add an antiseptic as formalin or 0.1% salicylic acid. Employ in same way as *carmalum.* Gives blue violet color. Very penetrating but not so specific for chromatin as carmalum (Lee, p. 142).

**Alveolar Epithelium of Lungs**

1. Gold sodium thiosulphate (Bensley, R. D. and S. H., Anat. Rec., 1935, **64,** 41–49). Inject a mouse intravenously through the tail vein with 100 mg. of gold sodium thiosulphate in 1 cc. aq. dest. The mouse dies in about 20 min. from asphyxia. Fix pieces of lung in 10% neutral formalin, dehydrate without washing in water, clear and imbed in paraffin. Deparaffinise sections and stain in 1% aq. toluidin blue and examine in water. The epithelium is raised by increase in volume of ground substance which is stained metachromatically pink while the cells and their nuclei are blue. The color of the ground substance can be changed to blue by alcohol and back again to pink by water. To mount protect against reversing action of alcohol by treating with equal parts 5% aq. ammonium molybdate and 1% aq. potassium ferrocyanide. Dehydrate, clear in xylol and mount in

2. Silver nitrate (Bensley, R. D. and S. H., Anat. Rec., 1935, **64,** 41–49). Use guinea pigs. *Silver Citrate* sol. (which see) is injected into lung substance by hypodermic syringe, the roots of the lung being first ligated, until the lung is moderately distended. Cut out pieces, fix in 10% formalin, embed in paraffin or celloidin, section, develop with dilute photographic developer and counterstain or examine unstained. The margins of the cells are blackened.

**Alveolar Pores** of the lung (Macklin, C. C. Arch. Path., 1936, **21,** 202–216). Formalin (10%) and Zenker-formalin are

among the fixatives suggested. The fixative is injected into the trachea or bronchus at a gravity pressure of 4–6 inches until the lungs are moderately distended. During this operation they are covered with physiological salt solution. The lungs are then immersed in fixative for days or even weeks. Slices about 1 cm. thick are cut, imbedded in soft paraffin and sections are made at 100$\mu$ or more. *Resorcin-fuchsin* and other stains may be used. The blood in the capillaries is a useful guide. The pores can be identified by their rounded edges.

**Alzheimer's Modification** of Mann's eosin-methyl blue for neuroglia and degenerate nerve fibers as given by Mallory (p. 245) is abbreviated. Fix thin slices, 14 days, in Weigert's **Neuroglia Mordant** + 10% of formalin. Wash 8–12 hrs. in running water. Mordant 10$\mu$ frozen sections 2–12 hrs. in sat. aq. phosphomolybdic acid. Wash 2 changes aq. dest. Stain in **Mann's Eosin Methyl Blue** 1–5 hrs. Wash quickly in aq. dest. until color "clouds" are no longer given off. Treat with 95% alcohol until gray matter becomes light blue and white matter pink or bright red. Dehydrate quickly in absolute alcohol, clear in xylol and mount in balsam. Normal axis cylinders, purple or deep blue; degenerating ones, red; neuroglia fibers, dark blue; and neuroglia cytoplasm, pale blue. Mallory states that change from blue to red staining of axis cylinders occurs as soon as 48 hrs. after experimental lesion.

**Amanil Garnet H.,** see **Erie Garnet B.**

**Amaranth** (CI, 184)—azo rubin, Bordeaux, Bordeaux SF, fast red, naphthol red S, C or O, Victoria rubin O, wool red—An acid mono-azo dye used long ago by Griesbach, H., Zeit. wis. mikr., 1886, **3,** 358–385 to color axis cylinders.

**Amebae,** see **Endamoeba.**

**Amethyst Violet** (CI, 847)—heliotrope B, iris violet—It is a basic azin dye of little importance to histologists.

**Amino Acids,** see **Alloxan Reaction,** also Schmidt, C. L. A., The Chemistry of the Amino Acids and Proteins. Springfield, Charles C. Thomas, 1938, 1031 pp.

**Aminoacridines,** some are strong antiseptics, do not stain skin (Albert, A. and Ritchie, B., J. Soc. Chem. Ind., 1941, **60,** 120).

**Amitosis** is direct nuclear division by constriction without formation of a chromatin thread. No special technique required. Study of embryonic membranes and of bladder of mouse (Dogiel, A. S., Arch. f. Mikr. Anat., 1890, **35,** 389–406) is suggested.

**Ammonia Carmine** (Ranvier). A suspension of carmine in water, with slight excess ammonia, is allowed to evaporate in air. If it putrefies so much the better. Dissolve the dry deposit in aq. dest. and filter (Lee, p. 145).

**Ammonium** molybdate, as mordant for Mann's stain and Weigert-Pal (Perdrau, J. R., J. Path. & Bact., 1939, **48,** 609–610).

**Amphinucleolus** (G. *amphi* on both sides). A nucleus which is double consisting of both acidophilic and basophilic parts, the former is usually a central core and the latter plastered on its surface.

**Amphophilic,** see **Staining.**

**Amyl Acetate,** as solvent for imbedding tissues (Barron, D. H., Anat. Rec., 1934, **59,** No. 1 and Suppl., 1–3); as a clearing agent for embryological material (Drury, H. F., Stain Techn., 1941, **16,** 21–22).

**Amyl Alcohol.** Merck lists 3, commercial, normal and tertiary. It mixes with 95% alcohol and with xylol. Hollande (A. C., C. rend Soc. de Biol., 1918, **81,** 223–225) was the first to recommend amyl alcohol as a substitute for absolute alcohol in the dehydration of specimens stained by the Romanovsky and Giemsa techniques.

**Amyl Nitrite.** McClung (p. 620) says that this may serve as a dilator of peripheral capillaries when a complete injection of small blood vessels is required. Add it to the ether at time of anesthetization.

**Amylase,** micromethod for (Pickford, G. E. and Dorris, F., Science, 1934, **80,** 317–319). This was later used with marked success by Dorris (F., J. Exp. Zool., 1935, **70,** 491–527) in a study of relation between enzyme production and histological development of gut of amblystoma. An extract is made, adjusted to proper pH, applied to slides coated with a starch-agar solution and incubated. The slides are then washed, the coating fixed in formalin and colored with dilute iodine solution. Sites of amylase activity are clear or pink staining spots. For necessary details, see author's description. van Genderen and Engel (H. and C., Enzymologia, 1938, **5,** 71–80) localized this enzyme by analysis of horizontal sections through the intestinal wall. It was found that it is present in rabbits in maximum amounts in Brunner's glands. Holtér and Dogle (C. R. Lab. Carlsberg, Sér. Chim., 1938, **22,** 219–225) observed that in amebae it is concentrated in association with the mitochondria which they assume to be carriers of amylase. See Barnes, J. M., Brit. J. Exp. Path., 1940, **21,** 264–275 for identification of amylase in lymphocytes and polymorphonuclear leucocytes.

**Amyloid** (G. *amylon,* starch and *eidos,* re-

semblance), a substance which accumulates in pathological conditions in the tissue fluids between cells particularly in chronic infections. Methods for its detection are fully described by Mallory and Parker (McClung, pp. 417-419). From numerous tests the following are selected:

1. *Iodine and sulphuric acid:* Stain section lightly with Lugol's iodine. Place in 1–5% aq. or conc. sulphuric or hydrochloric acid. Color of amyloid changes quickly from red through violet to blue or it may become deep brown.

2. *Methyl-violet:* Treat frozen sections of fresh, formalin or alcohol fixed tissue with 1% aq. methyl violet, 3–5 min. Wash in 1% aq. acetic acid, and remove acid by washing carefully in water. Examine in glycerin or water. Amyloid is violet and tissue blue. Colors will be retained longer if sections are mounted in **Levulose Syrup.**

3. *Iodine green:* Fresh or hardened sections are stained 24 hrs. in 0.3% aq. iodine green. Wash in water and examine in water or glycerin. Amyloid is stained violet red and tissue, green.

4. *Mayer's stain:* Transfer paraffin sections immediately after cutting to 0.5% aq. methyl violet or gentian violet at 40°C. for 5–10 min. Rinse in water and differentiate in 1% aq. acetic acid for 10–15 min. Wash thoroughly in water. Change to ½ sat. aq. alum and wash it off in water. Place section on slide and let water evaporate. Remove paraffin, clear in xylol and mount in balsam. Crystal violet and iodine green can be employed in the same way.

A Congo red test has been described (Taran, A., J. Lab. & Clin. Med., 1936–37, **22**, 975–977) and a polysaccharide has been isolated from amyloid bearing tissues which closely resembles chondroitin-sulphuric acid obtained from infantile cartilage (Hass, G., Arch. Path., 1942, **34**, 92–105).

**Anaplasma** is a small spherical body found within red blood cells in anaplasmosis diseases. There are two types *A marginale* and *A centrale* depending upon whether the bodies are situated near the margin or in the centers of the cells. The bodies are supposed to be parasites consisting of nuclear material with little if any cytoplasm. Anaplasmosis is important economically as a group of tick borne diseases of domestic animals. For demonstration stain blood smears by the methods of **Giemsa** or **Wright.**

**Anethol** is anise camphor suggested as a medium in which to soak tissues before making frozen sections (Stephanow, Zeit. wiss. Mikr., 1900, **17**, 181).

**Angstrom Unit.** 1Å = 0.1 mμ = 0.0001μ = $10^{-7}$ mm.

**Anhydrase,** see **Carbonic Anhydrase.**

**Anilin Blue Alcohol Soluble,** see **Spirit Blue.**

**Anilin Blue,** WS (CI, 707)—China blue, cotton blue, marine blue V, soluble blue 3M or 2R, water blue (Wasserblau)—A mixture of trisulphonates of di-phenyl rosanilin and tri-phenyl pararosanilin. Conn (p. 135) explains that this designation (like acid fuchsin) applies not to a single compound but to a group of dyes. Anilin blue is, nevertheless, the best stain for **Collagenic Fibers** and is employed for many other purposes.

**Anilin Crystal Violet** 1. **Ehrlich's.** Shake up 5 cc. anilin oil with 95 cc. aq. dest., Filter and to 84 cc. of filtrate add 16 cc. sat. alc. crystal violet. Leave 24 hrs. before using. After about 10 days staining potency decreases (Mallory, p. 89).

2. **Stirling's.** Crystal violet, 5 gm.; abs. alc., 10 cc.; anilin oil, 2 cc., aq. dest., 88 cc. Keeps well (Mallory, p. 90).

See **Anilin Crystal Violet** and **Gentian Violet.**

**Anilin-Fuchsin Methyl Green** method for mitochondria. This technique is based on Altman's method. It was used by Bensley to stain tissues fixed in his **Acetic-Osmic-Bichromate** fluid. Cowdry recommends instead fixation in the better penetrating Regaud's fluid.

Fix small pieces in freshly prepared Regaud's fluid (3% aq. potassium bichromate 4 parts, commercial formalin 1 part). Ordinarily it is not necessary to neutralize the formalin before hand by saturating it with magnesium carbonate. Keep in ice box and change the fluid every day for 4 days. Pour off fixative and mordant in 3% aq. potassium bichromate 8 days changing every second day. Wash in running water over night or in several changes of water. Dehydrate in alcohol, clear in xylol, imbed in paraffin and cut sections about 4 μ thick. Pass mounted sections through xylol and alcohol to water. Dry the slide with a cloth except area covered by sections. Pour on anilin acid fuchsin and heat to steaming over a spirit lamp. (To make this saturate 125 cc. aq. dest. with anilin oil by shaking the two together. Filter and add 15 gms. acid fuchsin to 100 cc. of filtrate. Allow to stand 24 hrs. before using. It lasts about a month.) Allow to cool and stain about 6 min. Pour stain back into bottle. Remove most of remainder, except from sections, with a cloth or filter paper. Rinse in aq. dest. about 1 min. Allow 1% aq. methyl green, added with a dropper, to flow over sections and counter stain them. This usually takes about 5 sec. but the time must be determined by trial. Wash off excess methyl green in 95% alcohol, dehydrate quickly in absolute, clear in toluol

(or xylol) and mount in balsam. The mitochondria are stained crimson and the nuclei green. For colored illustrations see Cowdry, E. V., Contrib. to Embryol., Carnegie Inst. of Washington 1917, No. 11, 27–43. If the methyl green does not stain intensely enough treat the sections, before coloration with fuchsin, with 1% aq. potassium permanganate 30 sec. followed by 5% oxalic acid 30 sec. and wash in water. More methyl green can be retained by blotting the sections after staining in it with filter paper and by then passing directly to absolute alcohol. If the time of fixation and mordanting is reduced much below that specified the fuchsin itself may not color with sufficient intensity. Such preparations hold their colors for a year or more unless they have been unduly exposed to sunlight, or the balsam is acid.

**Anilin Fuchsin Picric Acid,** see **Altmann's method** for mitochondria.

**Anilin Fuchsin Toluidine Blue** and **Aurantia,** see **Champy-Kull** method for mitochondria.

**Anilin Gentian Violet** usually credited to Ehrlich. Rarely is its composition given exactly the same by any two people. The "emended formula" (Soc. Am. Bact.) is A: 2.5 gm. crystal violet (85 per dye content) + 95% ethyl alcohol, 12 cc. B: anilin oil 2 cc. + aq. dest. 98 cc. (shake, leave few minutes, filter). Mix A and B. (McClung, p. 137).

**Anilin Oil.** A good product is easily obtainable. It is much used in the making of stains (cf. anilin fuchsin) and to clear tissues from 95% alcohol and even sections from 70%. Lee (p. 71) says that it should not be employed after fixation in osmic acid and that unless removed by chloroform or xylol it will give the tissues and mounting medium a brown coloration.

**Anilin Red,** see **Basic Fuchsin.**

**Anilin-Safranin** (Babes). Aq. dest., 98 cc.; anilin oil, 2 cc.; excess of safranin O. heat in flask in hot water bath at 70–80°C. Cool, filter and use filtrate.

**Anterior Chamber of Eye.** This is in many respects the best site for observations on transplanted tissues. See transplantation of uterine mucosa (Markee, J. E., Contrib. to Embryol., Carnegie Inst. of Washington, 1940, **28,** 219–308) and of tumors (Saphir, O., Appel, M. and Strauss, H., Cancer Res., 1941, **1,** 545–547). Aqueous humor is not so species specific as other tissue fluids that have been investigated. Consequently transplants from other species will often develop. There is of course the advantage of direct observation through the transparent cornea.

**Anthraquinone Dyes.** Derivatives of anthracene through anthraquinone. Acid alizarin blue GR and BB, alizarin, alizarin red S, purpurin.

**Anticoagulant Solutions** have been very carefully studied by Leichsenring, J. M., et al., J. Lab. & Clin. Med., 1939–40, **25,** 35–44. They found that 1.6% potassium oxalate prepared from dried salt is most nearly isotonic for human blood. Wintrobe, M. M., Clinical Hematology, Philadelphia, Lea & Febiger, 1942, 792 pp. advises 0.06 gms. of ammonium oxalate and 0.04 gms. of potassium oxalate for 5 cc. of blood. He dissolves 1.2 gm. ammonium oxalate and 0.8 gm. potassium oxalate in 100 cc. aq. dest. and adds 1 cc. formalin to prevent deterioration. Then he measures out with a burette 0.5 cc. into each of the containers and lets it dry before taking into each 5 cc. of fresh blood. *Heparin* is also advised but it is much more expensive. 0.075 gm. will prevent coagulation of 5 cc. of blood. See **citrate.**

**Antimony Trichloride,** see **Carr-Price Reaction.**

**Aorta,** see **Arteries** and, for an account of technique for measuring elastic properties, Saxton, J. A., Arch. Path., 1942, **34,** 262–274.

**Aortic Paraganglion** (Glomus aorticum). Technique for blood supply and innervation is provided by Monidez, J. F., J. Anat., 1936, **70,** 215–224. Negative results in application of the chromaffin reaction to the rabbit and guinea pig are described by the same author, Am. J. Anat., 1935, **57,** 259–293. Carotid glomus is very similar.

**Arachnids,** sectioning is facilitated by methods intended to soften **Chitin.** See also **Fleas, Ticks.**

**Archelline 2B,** see **Bordeaux Red.**

**Argentaffine** gastrointestinal cells (enterochromaffin cells). Rare even in duodenum. Occur singly, usually in deepest parts of crypts and may be free from epithelium. Cytoplasmic argentaffine granules are of small size, often closely packed together and acidophilic. It is said that they cannot be found in bodies autopsied as late as 4–5 hrs. after death (Hamperl, H., Ztschr. f. Mikr.-anat. Forsch., 1925, **2,** 506–535).

Two specific methods are advised by Jacobson, W., J. Path. & Bact., 1939, **49,** 1–19. For both fix in 10% formol-saline, or 10% neutral formol, dehydrate in alcohol, clear in cedarwood oil or in methyl benzoate + 2% celloidin and imbed in paraffin. In the first wash deparaffinized sections 10 mm. in 2 changes glass-dist. water. Transfer for 12–24 hrs. to Fontana's sol. prepared by adding $NH_4OH$ to 5% $AgNO_3$ until ppt. is dissolved, then $AgNO_3$ drop by drop until fluid exhibits slight presistent opalescence. Wash in glass-dist. water, 1 min., 5% $Na_2S_2O_3$, 1 min. and tap

water 10 min. Counterstain with carmalum. Dehydrate, clear and mount in balsam. Granules of argentaffine cells appear black. In the second more rapid method dissolve small amount p-nitro-methyloxybenzene diazotate in aq. dest. producing light yellow solution alkalinize with a little $Li_2CO_3$. After about 1½ min., when pH 10–11 is reached, color has changed to dark orange-yellow. Immerse sections brought down to aq. dest., in this 30–40 sec. Then wash in aq. dest., 1 min. Granules of argentaffine cells appear dark red in yellow background. Counterstain with hemalum if desired.

**Argentaffine Reaction.** This, according to Lison (p. 147) is given by polyphenols, aminophenols and polyamines in ortho and para position. It is a reduction of ammoniated silver hydroxide into metallic silver. He recommends Masson's method for sections: Fix in Bouin's fluid or other fixative. Deparaffinize sections and wash 2 hrs. in aq. dest. Treat for 36–40 hrs. in Fontana's fluid in darkness and in a sheltered place. Wash in much aq. dest. Tone with 0.1% aq. gold chloride (few minutes). Fix in 5% aq. sodium hyposulphite. Counterstain with alum carmine, mount in usual way. To make Fontana's fluid add ammonia drop by drop to 5% aq. silver nitrate until ppt. formed is exactly redissolved; then carefully drop by drop 5% aq. silver nitrate until appearance of persistent cloudiness and the liquid does not smell of ammonia. Decant before employing. See also Clara, M., and Canal, F., Zeit. f. Zellf. u. Mikr. Anat., 1932, **15**, 801–808; Clara, M., Ergeb. d. Anat. n. Entw., 1933, **30**, 240–340.

**Arginase.** It is possible to localize arginase in the cytoplasm and nuclei of liver cells by Behren's technique (Zeit. Physiol. Chem., 1939, **258**, 27–32). Finely ground tissue is dried to powder in frozen condition. It is then suspended and centrifuged in different mixtures of benzene and carbon tetrachloride. The nuclei only are found in the lowest layer, next comes nuclear debris and above this cytoplasmic debris. His analysis showed arginase present in the same concentration in the nuclei as in the cytoplasm. Blaschko and Jacobson (Bourne, p. 217) remark that this is the first instance of the demonstration of an enzyme in the cell nucleus.

**Argyrophilic Fibers.** Because of their affinity for silver, **Reticular Fibers** are often called argyrophilic.

**Arsenic** 1. Use 10% neutral formalin in aq. dest. after test with hydrogen sulphide shows absence of trace of metals. To 100 cc. add 2.5 gm. copper sulphate. Fix small pieces of tissue 5 days. Wash 24 hrs. in running water. Imbed in paraffin. Direct examination of section after removal of paraffin shows arsenic as well defined green granules of hydroarsenite of copper (Scheele's green). If neutral acetate of copper is employed in place of the sulphate the green granules are of acetoarsenite of copper (Schweinfurth's green).

2. Fix pieces of tissue 12–24 hrs. in abs. alc. 50 cc.; chloroform, 50 cc.; pure hydrochloric acid, 3 cc. saturated by passage of pure hydrogen sulphide. In sections the arsenic ppt. appears as yellow granules. Double coloration with hematein-eosin is possible. Both techniques have been devised by Castel (P., Bull. d'Hist. Appl., 1936, **13**, 106–112). He has described the histologic distribution of the arsenic. See, however, paper by Tannenholz, H. and Muir, K. B., Arch. Path., 1933, **15**, 789–795 who employed a somewhat similar method and were unable to conclude that the yellow crystals were in fact those of arsenic trisulphide. They considered them more probably a sulphur-protein combination.

The distribution to the several tissues of radioactive arsenic injected intravenously into rabbits as sodium arsenate has been investigated by duPont, O., Irving, A. and Warren, S. L., Am. J. Syph. etc., 1942, **26**, 96–118. It is important to determine whether the results conform with those given by the microchemical techniques.

**Artefacts,** see **Artifacts.**

**Arteries.** If one wishes an *elastic* artery take a large trunk near the heart such as the aorta, innominate or subclavian; if, on the other hand, a typical *muscular* artery is required select one further afield like the radial or external carotid. Arterial walls are seldom examined microscopically *in vivo* because they are relatively large and difficult to get at without injury. An exception in man is the retinal artery which can be seen by ophthalmoscopic examination. To closely observe excised pieces of arteries is all too frequently neglected. The tissue elements are so tightly bound together that to tease them apart for study at high magnification is rather unsatisfactory. However, when the adventitial adipose and connective tissue is stripped off from a fresh specimen, the remainder of the wall can very advantageously be made translucent by treatment with pure glycerin for 1–2 hrs. as described by Winternitz, M. C., Thomas, R. M. and LeCompte, P. M. in their book "The Biology of Arteriosclerosis", Springfield: Thomas, 1938, 142 pp. Since the color of the blood is preserved within the intramural vessels their arrangement can be studied (see **Vasa Vasorum**).

Fatty substances can also be located because they are not removed by the glycerin.

Chief reliance is ordinarily placed in the appearance of arterial walls when seen in sections of fixed tissue. It is important to remember that, when carrying blood during life, the lumina are larger and the walls less folded than in the fixed condition. The difference has been graphically demonstrated by Galloway, R. J. M., Am. J. Path., 1936, **12**, 333–336. His figures should be examined. For routine purposes fixation in **Formalin-Zenker** followed by **Mallory's Connective Tissue Stain** supplemented by **Resorcin Fuchsin** or **Orcein** for elastic tissue is satisfactory. Special methods may be needed for **Lipids**; and for minerals, see **Calcium, Iron** and **Microincineration.** Innervation, likewise, is to be studied by methods employed to demonstrate **Nerve Endings** in other tissues. See **Vasa Vasorum.** Much literature on techniques is given by various authors in Cowdry, E. V., Arteriosclerosis, New York: Macmillan, 1933, 617 pp.

**Arterioles,** capillaries and venules, in contrast to the much larger arteries and veins, can readily be examined in experimental animals microscopically in the living state. Since they are linked together a single preparation by **Sandison's** rabbit ear method shows all three, or they may be viewed in the living tadpole's tail or other transparent tissue of lower forms. For convenience, however, it seems best to briefly mention the microscopic techniques for each separately. There is much to choose from. Information is frequently demanded on the condition of the arteriolar walls. This can best be supplied by staining paraffin sections of **Formalin-Zenker** fixed material with **Mallory's Connective Tissue** stain or with **Masson's Trichrome** stain which is closely related to it. **Weigert's Resorcin Fuchsin** is satisfactory for elastic tissue. The **Silver Citrate** technique is capable of yielding valuable data on arterioles and capillaries. Because arterioles contain a higher percentage of muscle than any other blood vessel their appearance will vary greatly with the degree of contraction or relaxation of muscle. According to Kernohan, J. W., Anderson, E. W. and Keith, N. M., Arch. Int. Med., 1929, **44**, 395–423 in fixed preparations from normal persons the average ratio of thickness of arteriolar wall to width of lumen is 1:2.

**Arteriovenous Anastomoses** are direct connections between arteries and veins without intervening capillaries. No special histological technique is required for their demonstration in sections but one should look for them where they are particularly numerous, as in rabbits at the tip of the nose (diameter, 80–100$\mu$) and in humans in the palms of the hands, the soles of the feet and near the ends of the fingers where their diameter is about 35$\mu$ (Grant, R. T. and Bland, E. F., Heart, 1930, **15**, 385–411). The best way is to study them *in vivo* (Clark, E. R. and E. L., Am. J. Anat., 1934, **55**, 407–467).

**Articular Nerve Terminals.** Gardner, E. D., Anat. Rec., 1942, **83**, 401–419, working in our laboratory, has very successfully adapted silver methods to the demonstration of nerve terminals associated with the knee joints of mice. Knee joints of mice 1–60 days old were studied. Immerse joints of young animals in 10% formalin 1–2 days and of older ones in 20% formalin for the same time. Wash, dehydrate to 70% alcohol. Decalcify in 2.5% nitric acid in 70% alcohol, 2–12 hrs. Wash in 70% alcohol until neutral to blue litmus paper. Infiltrate with celloidin (amyl acetate 20 parts, acetone 40 and paralodion 10) taking care not to let too much enter the tissue. Harden in chloroform 5–10 min. Clear in xylol, half paraffin and xylol over night at 37°C. Imbed in paraffin and cut 10$\mu$ sections. Treat sections with 5% ammonium hydroxide in 80% alcohol before transferring to protargol (Winthrop) for 12–48 hrs. as in the **Bodian** technique. Axones black against reddish gray background. To obtain these results consistently chemically cleaned glassware and doubly distilled water are essential throughout.

**Artifacts.** Webster defines an artifact as being "in histology, a structure or appearance in a tissue or cell due to death or to the use of reagents and not present during life." The degree of artifact is proportional to the difference between the structure existing normally in the living body and the structure in the condition directly studied.

1. In the case of *living tissues*, observed with blood and nerve supply intact, there is a possibility of artifact. It is at a minimum in the **Rabbit Ear Chambers** and rather more to be reckoned with when tissues must be displaced in order to supply the necessary illumination. With increase in time modifications due to changes in light, temperature, hydrogen ion concentration, etc. are likely to also increase.

2. In *living cells* removed from the body and examined in **Tissue Cultures** the possibility of artifact is again at a minimum; but, though the cells in successive generations in suitable media go on living indefinitely, their environments are different from those existing within the body. When after **Vital**

**Staining** or **Supravital Staining** still living cells are examined in approximately isotonic media, there is a grave danger of artifact if the study is prolonged because the cells are slowly dying.

3. In *fixed tissues* the degree of divergence from the normal living condition is obviously much greater than in the case of still living ones. However death has been sudden so that artifacts due to gradual death are eliminated. If the technique has been carefully standardized the same fixative applied to the same type of cell in the same physiological state is likely to yield similar results. Among common artifacts are: 1. The shrinkage and increased affinity of cells near the surface for stains due to allowing the surface of the tissue to dry before fixation. 2. The glassy appearance of nuclei and cytoplasm sometimes occasioned by overheating in imbedding or in spreading out sections. 3. Material within blood vessels faintly resembling organisms caused by coagulation of blood proteins. 4. Extraneous substances either present in the albumen fixative used to mount the sections or deposited as dust from the air. Careful focussing is required. See also **Agonal** and **Postmortem** changes.

**Artificial Fever,** influence on adrenal (Bernstein, J. G., Am. J. Anat., 1940, **66,** 177–196). See Cramer, W., Fever, Heat Regulation and the Thyroid-Adrenal Apparatus. London: Longmans, Green & Co., 1928, 153 pp.

**Ascorbic Acid,** see **Vitamin C.**

**Aspirated Sternal Marrow,** method for preparing smears and sections (Gordon, H., J. Lab. & Clin. Med., 1940–41, **26,** 1784–1788).

**Atomic Weights.** The 11th Report of the Committee on Atomic Weights of the International Union of Chemistry. Baxter, G. P. (chairman) et al., J. Am. Chem. Soc., 1941, **63,** 845–850 gives the following as *International Atomic Weights, 1941.*

| Element | Symbol | Atomic Number | Atomic Weight |
|---|---|---|---|
| Aluminum | Al | 13 | 26.97 |
| Antimony | Sb | 51 | 121.76 |
| Argon | A | 18 | 39.944 |
| Arsenic | As | 33 | 74.91 |
| Barium | Ba | 56 | 137.36 |
| Beryllium | Be | 4 | 9.02 |
| Bismuth | Bi | 83 | 209.00 |
| Boron | B | 5 | 10.82 |
| Bromine | Br | 35 | 79.916 |
| Cadmium | Cd | 48 | 112.41 |
| Calcium | Ca | 20 | 40.08 |
| Carbon | C | 6 | 12.010 |
| Cerium | Ce | 58 | 140.13 |
| Cesium | Cs | 55 | 132.91 |
| Chlorine | Cl | 17 | 35.457 |
| Chromium | Cr | 24 | 52.01 |
| Cobalt | Co | 27 | 58.94 |
| Columbium | Cb | 41 | 92.91 |
| Copper | Cu | 29 | 63.57 |
| Dysprosium | Dy | 66 | 162.46 |
| Erbium | Er | 68 | 167.2 |
| Europium | Eu | 63 | 152.0 |
| Fluorine | F | 9 | 19.00 |
| Gadolinium | Gd | 64 | 156.9 |
| Gallium | Ga | 31 | 69.72 |
| Germanium | Ge | 32 | 72.60 |
| Gold | Au | 79 | 197.2 |
| Hafnium | Hf | 72 | 178.6 |
| Helium | He | 2 | 4.003 |
| Holmium | Ho | 67 | 164.94 |
| Hydrogen | H | 1 | 1.0080 |
| Indium | In | 49 | 114.76 |
| Iodine | I | 53 | 126.92 |
| Iridium | Ir | 77 | 193.1 |
| Iron | Fe | 26 | 55.85 |
| Krypton | Kr | 36 | 83.7 |
| Lanthanum | La | 57 | 138.92 |
| Lead | Pb | 82 | 207.21 |
| Lithium | Li | 3 | 6.940 |
| Lutecium | Lu | 71 | 174.99 |
| Magnesium | Mg | 12 | 24.32 |
| Manganese | Mn | 25 | 54.93 |
| Mercury | Hg | 80 | 200.61 |
| Molybdenum | Mo | 42 | 95.95 |
| Neodymium | Nd | 60 | 144.27 |
| Neon | Ne | 10 | 20.183 |
| Nickel | Ni | 28 | 58.69 |
| Nitrogen | N | 7 | 14.008 |
| Osmium | Os | 76 | 190.2 |
| Oxygen | O | 8 | 16.0000 |
| Palladium | Pd | 46 | 106.7 |
| Phosphorus | P | 15 | 30.98 |
| Platinum | Pt | 78 | 195.23 |
| Potassium | K | 19 | 39.096 |
| Praseodymium | Pr | 59 | 140.92 |
| Protactinium | Pa | 91 | 231 |
| Radium | Ra | 88 | 226.05 |
| Radon | Rn | 86 | 222 |
| Rhenium | Re | 75 | 186.31 |
| Rhodium | Rh | 45 | 102.91 |
| Rubidium | Rb | 37 | 85.48 |
| Ruthenium | Ru | 44 | 101.7 |
| Samarium | Sm | 62 | 150.43 |
| Scandium | Sc | 21 | 45.10 |
| Selenium | Se | 34 | 78.96 |
| Silicon | Si | 14 | 28.06 |
| Silver | Ag | 47 | 107.880 |
| Sodium | Na | 11 | 22.997 |
| Strontium | Sr | 38 | 87.63 |
| Sulfur | S | 16 | 32.06 |
| Tantalum | Ta | 73 | 180.88 |
| Tellurium | Te | 52 | 127.61 |
| Terbium | Tb | 65 | 159.2 |
| Thallium | Tl | 81 | 204.39 |
| Thorium | Th | 90 | 232.12 |
| Thulium | Tm | 69 | 169.4 |
| Tin | Sn | 50 | 118.70 |
| Titanium | Ti | 22 | 47.90 |
| Tungsten | W | 74 | 183.92 |
| Uranium | U | 92 | 238.07 |
| Vanadium | V | 23 | 50.95 |
| Xenon | Xe | 54 | 131.3 |
| Ytterbium | Yb | 70 | 173.04 |
| Yttrium | Y | 39 | 88.92 |
| Zinc | Zn | 30 | 65.38 |
| Zirconium | Zr | 40 | 91.22 |

**Auditory System,** see **Ear.**

**Auer Bodies.** A technique whereby red cells are overstained and leucocytes understained has proved helpful for the demonstration of these rod like bodies in leucocytes (Goodwin, A. F., Folia Haemat., 1933, **51,** 359–366). The smears are colored in the regular fashion by Wright's stain except that water is not added to the stain.

**Auerbach's Plexus.** Supravital staining by injecting methylene blue through the aorta is apparently improved by addition of hydrogen acceptors. Schabadasch, A., Bull. d'Hist. Appl., 1936, **13,** 1–28, 72–89, 137–151 advises 0.03–0.05 gm. per liter of *p*-amidophenol, 0.02–0.07 of *p*-phenylenediamine, 0.02–0.05 of pyrocatechine or 0.05–0.9 of resorcin. The methylene blue must be of high quality and free from metallic salts. He obtained in 5 min. intense staining of the plexus in a cat which received 1200 cc. of fluid of the following concentration: aq. dest., 1000 cc.; NaCl, 7 gm.; resorcin, 0.15 gm. and methylene blue (chlorzink free, Hoecht) 0.2 gm.

**Auramin** (CI, 655)—canary yellow, pyoktanin yellow, pyoktaninum aureum—This basic diphenyl methane dye may be of use in fluorescence microscopy. Auramine O is Commission Certified. It is one of the substances which arrests mitosis in the metaphase, an action which has been carefully studied by Ludford, R. J., Arch. f. Exper. Zellf., 1935–6, 18, 411–441. Tubercle bacilli treated with auramin give golden yellow fluorescence (Hageman, P. K. H., Munch. Med. Woch., 1938, 85, 1066).

**Aurantia** (CI, 12)—imperial yellow—An acid nitro dye employed in **Champy-Kull** method.

**Aurin** or rosolic acid (CI, 724).

**Axenfeld Reaction.** Giroud (A., Protoplasma, 1929, **7,** 72–98): Add to preparation few drops of formic acid, then 3–4 drops 0.1% aq. gold chloride and heat slowly. A rose color appears, then violet. Lison (p. 129) says that the reaction is very little characteristic of proteins since analogous reactions are given by creatine, urea, uric acid, glycogen. Its employment is contraindicated.

**Axis Cylinders.** These are the cytoplasmic cores of the nerve fibers. Mitochondria can often be seen in them unstained and after supravital coloration with **Janus Green.** The best method to demonstrate mitochondria in fixed tissues is **Anilin Fuchsin Methyl Green** after Regaud fixation. Silver methods show **Neurofibrils. Alzheimer's** modification of Mann's eosin-methyl blue method is recommended to show early degenerative changes. De Renyi, G. S., Cowdry's Special Cytology, 1932, **3,** 1370–1402 has fully described use of methods of microdissection. See **Amaranth.**

**Azan Stain,** see **Heidenhain's.**

**Azidine Blue 3B,** see **Trypan Blue.**

**Azidine Scarlet R,** see **Vital Red.**

**Azins.** Azin dyes are those formed from phenazin. Two benzene rings are joined by 2 nitrogen atoms forming a third ring. Examples: amethyst violet, azocarmine G, indulin alcohol and water soluble, Magdala red, neutral red, neutral violet, nigrosin water soluble, phenosafranin, safranin O.

**Azo Blue** (CI, 463)—benzoin blue R and direct violet B—This acid dis-azo dye is one of those microinjected vitally into cytoplasm against the nucleus of amebae to ascertain whether the nucleus can be vitally colored (Monné, L., Proc. Soc. Exp. Biol. & Med., 1934–35, **32,** 1197–1199). Butt, E. M., Bonynge, C. W. and Joyce, R. L., J. Inf. Dis., 1936, **58,** 5–9 report that azo blue can be substituted for India ink in the negative demonstration of capsular zones about hemolytic streptococci.

**Azo-Bordeaux,** see **Bordeaux Red.**

**Azocarmine G** (CI, 828)—azocarmine GX, rosazine, rosindulin GXF—This basic azin dye is used in place of acid fuchsin in **Heidenhain's Azan** stain. Azocarmine B is CI, 829.

**Azocarmine GX,** see **Azocarmine G.**

**Azo Dyes.** Chromophore—N=N—uniting naphthalene or benzene rings. See **Mono-azo, Dis-azo** and **Poly-azo Dyes.** Lipophilic substitutions in, and slight curative effect claimed in tuberculosis and leprosy (Bergmann, E., Haskelberg, L. and Bergmann, F., J. Am. Chem. Soc., 1941, **63,** 2243.

**Azo-fuchsin.** Seven are recognized in the Colour Index. Acid mono-azo dyes related to Bordeaux red and orange G.

**Azo Reaction** for phenols. Formation of azo color by action of diazonium salt on tissue phenol (Lison, p. 140). See Lison, L., C. Rend. Soc. de Biol., 1933, **112,** 1237–1239).

**Azo Rubin,** see **Amaranth.**

**Azure Dyes.** These are basic thiazin stains of great usefulness. The description given by Conn (pp. 76–80) should be consulted. It is here summarized. Azure I (Giemsa) is a trade name for a secret preparation apparently a variable mixture of Azure A and B. Azure II is an intentional mixture, in equal parts, of Azure I and methylene blue. It is the main constituent of **Giemsa's** stain.

1. *Azure A* is asymmetrical dimethyl thionin and has been Commission Certified for some time. It is considered as the most important nuclear staining component of polychrome methylene blue by MacNeal, W. J., J. Inf. Dis., 1925, **36,** 538–546. This dye has been used as a nuclear stain following eosin

and after phloxine, see **Phloxine-Azure** (Haynes, R., Stain Techn., 1926, **1**, 68–69, 107–111).

2. *Azure B* is the tri-methyl derivative of thionin. It is specified by Jordan, J. H. and Heather, A. H., Stain Techn., 1929, **4**, 121–126 as a stain for Negri bodies. Roe, M. A., Lillie, R. D. and Wilcox, A., Pub. Health Reports, 1940, **55**, 1272–1278 recommend its inclusion in Giemsa's stain.

3. *Azure C* is mono-methyl thionin. French, R. W., Stain Techn., 1926, **1**, 79 has described a method for its use followed by Eosin Y and orange II in staining sections of formalin fixed material; but Haynes, R., Stain Techn., 1927, **2**, 8–16 doubts whether it is significantly better than Azure A and thionin.

**Azure II Eosin and Hematoxylin** (Maximow, A., J. Inf. Dis., 1924, **34**, 549), gives, in addition to coloration of chromatin by hematoxylin, a granule stain something like that provided by Giemsa's method. Make up: (1) *azure II eosin*: A. eosin water soluble yellowish, 0.5 gm.; aq. dest., 500 cc. B. azure II, 0.5 gm.; aq. dest., 500 cc. Mix 10 cc. A, 100 cc. aq. dest., and 10 cc. B. (2) *hematoxylin* (Delafield's) 1–2 drops, aq. dest., 100 cc. to make a pale violet solution.

Formalin-Zenker fixed tissues (sections, smears, spreads) are stained upright in hematoxylin washed in aq. dest. and counter-stained with azure II eosin 24 hrs. each. Transfer to 95% alc., differentiate and dehydrate in abs. (2 changes); clear in xylol and mount in balsam. Care must be taken to use pure aq. dest. The proportions of A and B can be varied slightly to suit the tissue. In order to hold the azure II eosin colors the balsam should be neutral or nearly neutral as when Giemsa's stain is employed.

To appreciate the beauty of this method see numerous colored illustrations marked "ZF, Häm, EAz" of a great many organs and tissues by Maximow, A. Section on Bindegewebe und Blutbildende Gemebe in Möllendorff's Handb. d. mikr. Anat. d. Menschen, 1927, **2**, (1) 232–583.

**Bacillus Typhosus,** technique for dark field study of flagella (Pijper, A., J. Path. & Bact., 1938, **47**, 1–17). See 9 plates by author.

**Bacteria.** Methods employed for the microscopic identification of bacteria and to demonstrate their structure are legion. The Committee on Bacteriological Technique of the Society of American Bacteriologists has prepared a useful leaflet entitled "Staining Procedures" published in Geneva, N. Y. (Fifth Edition 1934) to supplement their "Manual of Methods for the Pure Culture of Bacteria" (1923). A detailed account of Bacteriological methods by H. J. Conn, F. B. Mallory and Frederic Parker, Jr., is contained in McClung's Microscopical technique to which reference should also be made. Bergey's "Manual of Determinative Bacteriology", (Baltimore: Williams & Wilkins, 1925, 462 pp.), which is a key to identification of bacteria, is often useful.

Motility, agglutination, lysis under influence of bacteriophage, ingestion by leucocytes and many other phenomena can best be observed by examination of living bacteria by direct illumination or in the darkfield. Smears, usually fixed by heat, are, however, most often used. A choice must be made from many well known stains including: **Anilin Gentian Violet, Loeffler's Methylene Blue, Giemsa, Gram** and **Carbol Fuchsin.** Others are best listed under the particular structures to be demonstrated: **Spores, Flagella, Capsules.** In some cases search for bacteria in **Milk, Soil, Cheese, Sputum,** etc. is indicated. When bacteria are so few in number that they may be missed, or large numbers are required separated from the tissues for chemical analysis, **Concentration** methods may be useful. Accurate localization of bacteria requires their study in sections. See **Giemsa's** stain, **Gram-Weigert** stain, **Goodpasture's** stain (MacCallum's modification), Mallory's **Phloxine-Methylene** blue and **Acid Fast Bacilli.** The darkfield examination of stained preparations is said to be an advantage (Goosemann, C., J. Lab. and Clin. Med., 1935–36, **21**, 421–424). Appearance when viewed at high magnification with electron microscope (Mudd, S., Polevitsky, K., and Anderson, T. F., Arch. Path., 1942, **34**, 199–207). See **Fluorescence** microscopy, **Dead** bacteria, **Tubercle** bacilli, **Leprosy** bacilli, **Mitochondria** and **Bacteria** in same cells, **Rickettsia, Gonococcus, Diphtheria Bacilli.**

**Bacterial Pigments.** These cannot be measured microscopically but a method has been devised for doing so with spectrophotometer and photoelectric colorimeter (Stahly, G. L., Sesler, C. L. and Brode, W. R., J. Bact., 1942, **43**, 149–154).

**Bacterial Polysaccharides.** Solutions of reduced bases and leuco bases of penta- and hexa-methyl triamino-triphenylmethane and tetramethyl diamino-triphenylmethane and certain other triphenylmethanes react with staphylococcal polysaccharides and may be

useful in their detection (Chapman, G. H. and Lieb, C. W., Stain Techn., 1937, **12**, 15–20).

**Bacteriostatic Titration of Dyes.** (Reed, M. V. and Genung, E. F., Stain Techn., 1934, **9**, 117–128).

**Bacterium Monocytogenes.** Intravenous injections of this organism in rabbits produce a marked increase in the number of circulating monocytes and therefore provide an important experimental method (Murray, E. G. D., Webb, R. H. and Swan, M. B. R., J. Path. and Bact., 1926, **29**, 407–439).

**Bacterium Tularense** in sections. Add 10 cc. sat. aq. nile blue sulphate and 6 cc. 1% aq. safranin to 60 cc. aq. dest. Stain sections over night. Wash quickly, dehydrate in alcohols, clear in xylol and mount (Foshay, L., J. Lab. & Clin. Med., 1931, **17**, 193–195).

**Balsam** for mounting sections is usually satisfactory as purchased. To make, mix equal parts dry balsam and sodium bicarbonate and grind in mortar. Add sufficient xylol to make clear solution. After few days filter and heat gently (avoiding flame) to bring to suitable consistency. The best mounting medium when neutrality is essential is **Clarite** or the cedar oil used for oil immersion objectives. The latter sets more slowly than balsam and it is ordinarily not necessary to employ it. See **Mounting Media.**

**Barium,** spectrographic analysis of, in retina (Scott, G. H. and Canaga, B., Jr., Proc. Soc. Exp. Biol. & Med., 1940, **44**, 555–556). *Barium chloride* and formalin are advised as fixative for **Bile Components.** *Barium sulphate* emulsion injections are recommended by Woollard, H. H. and Weddell, G., J. Anat., 1934–35, **69**, 25–37 to demonstrate arterial vascular patterns. The emulsion should be of such consistency that it cannot easily be forced beyond the small arterioles by a pressure of 1.5 atmospheres. Fix tissues by hypodermic injection of formalin and subsequent immersion in it. Take x-ray photographs of the radiopaque barium.

**Basal Bodies** of cilia (Wallace, H. M., Science, 1931, **74**, 369–370). Fix in Zenker (containing acetic) or in Zenker-formalin (90 cc. Zenkers + 10 cc. 10% formalin). Mount paraffin sections 5$\mu$ thick. After very light staining with hematoxylin and thorough washing in tap water dip in 0.5% aq. eosin (Grubler's *wasserlich*. If not available, use **Eosin Y.**) ½ min. and wash quickly in large volumes of water. Make up stain by adding 9 parts sat. aq. methyl violet (Grubler's 6B only. If not available, use C.C. which is 2B.) to 1 part abs. alcohol 33 cc.; aniline oil 9 cc. + methyl violet in excess. Stain is best 3–8 days after mixing but the two solutions can be kept separately. After staining sections for 2 hrs. wash well in tap water, treat with Lugol's iodine 10–15 min. and repeat the washing. Blot with filter paper. Differentiate in 1 part aniline oil + 2 parts xylol. Wash in several changes of xylol and mount in balsam. Basal bodies deep purple, nuclei dark blue. Good also for intracellular bacteria and fibrin.

**Basic Brown, G, GX,** or **GXP,** see **Bismark Brown Y.**

**Basic Dyes,** see **Staining.**

**Basic Fuchsin**—anilin red, basic rubin, and magenta (CI 676 or 677)—Commission Certified. The tri-amino tri-phenyl methane dyes bearing this name are mixtures of pararosanilin, rosanilin and magenta II in varying proportions. They are employed for a great many purposes. New fuchsin (CI 678) is a different compound. It is the deepest in color of 4 dyes and pararosanilin is the lightest.

**Basic Lead Acetate** used as fixative for **Tissue Basophiles.**

**Basic Rubin,** see **Basic Fuchsin.**

**Basophila Erythroblasts,** see **Erythrocytes,** developmental series.

**Basophile Leucocyte** (mast-leucocyte, blood mast cell). Least numerous granular leucocyte; percentage about 0–1; slightly smaller (8–10$\mu$) than other types; nucleus spherical or slightly lobated, faintly staining and centrally placed; specific granules only slightly refractile, basophilic, large, variable and less numerous than in other types; function unknown. This cell is difficult to study in fresh preparations of peripheral blood because it is so scarce. Smears colored by the usual methods (Giemsa, Wright, etc.) are satisfactory. The basophilic granules appear to be particularly soluble in water. Doan and Reinhart (C. A. and H. L., Am. J. Clin. Path., 1941, **11**, Tech. Suppl. **5**, 1–39, with beautiful colored plates) recommend supravital staining with neutral red and janus green. There is difference of opinion as to whether the oxidase and peroxidase reactions are positive (Michels, N. A. in Downey's Hematology, 1938, **1**, 235–372). See **Tissue Basophiles.**

**Basophilic,** see **Staining.**

**Bell's Method** for fixing and staining of fats as described by the Bensleys (p. 114). Intracellular fats are mobilized by heat to form droplets which are chromated and later stained. Consequently the preparations show these fats, in addition to other microscopically visible fat, but not their true distribution in the cells.

Fix for 10 days at 45–50°C. in 10% aq. potassium bichromate 100 cc. + 5 cc. acetic acid. Imbed and make paraffin sections as usual. Pass them down to absolute alcohol. Stain with freshly prepared Sudan III 10 min. Rinse off in 50% alcohol and pass to water to arrest action of alcohol. Counter-stain with **Delafield's Hematoxylin.** Wash in water, differentiate in acid alcohol, wash in water again and mount in **Glycerine Jelly.**

**Benda's Method** of crystal violet and alizarin for mitochondria. Fix in Flemming's fluid 8 days (see **Flemming's Fluid**). Wash in water 1 hr. Then half pyroligneous acid and 1% chromic acid, 24 hrs. 2% potassium bichromate, 24 hrs. Wash in running water 24 hrs. Dehydrate, clear, imbed in paraffin and cut sections at 4$\mu$. Pass down to water and mordant in 4% iron alum 24 hrs. Stain amber-colored sol. sodium sulphalizarinate made by adding sat. alc. sol. to water, 24 hrs. Blot with filter paper and color in equal parts crystal violet sol. and aq. dest. (The sol. consists of sat. crystal violet in 70% alc. 1 part, alc. 1 part and anilin water 2 parts.) Warm until vapor arises and allow to cool 5 min. Blot and immerse in 30% acetic acid 1 min. Blot, plunge in abs. alc. until but little more stain is extracted, clear in xylol and mount in balsam. The mitochondria are stained deep violet in a rose background. The colors are more lasting than in Altmann preparations. This is one of the classical techniques of histology but it is difficult. For colored illustrations see Duesberg, J., Arch. f. Zellforsch., 1910, 4, 602–671.

**Benda's** stain for fat necrosis. See **Fischler's** modification.

**Bensley's Neutral Safranin.** For mitochondria and secretion antecedents especially in the pancreas. Fix in 2.5% aq. potassium bichromate, 100 cc.; mercuric chloride, 5 gms. 24 hrs. Wash, dehydrate, clear, imbed and section. To prepare stain slowly add sat. aq. acid violet to sat. aq. safranin O in a flask until ppt. ceases when a drop of mixture on filter paper gives not an outside red rim of safranin but a solid neutral color. Filter. The filtrate should be as nearly as possible colorless. Dry ppt. on filter paper and make of it a sat. sol. in absolute alcohol. Pass sections through 2 changes each of toluol and absolute alcohol, then down through lower alcohols to aq. dest. (Bleach chrome and osmium fixed tissues in permanganate and oxalic acid, as described under **Anilin Fuchsin Methyl Green** and mercury fixed ones in Lugol's solution, 10 sec. finally washing in aq. dest.) Dilute alcoholic neutral safranin with equal volume aq. dest. and stain 5 min.–2 hrs. Quickly blot with filter paper. Plunge into acetone and immediately pass to toluol without draining. Examine and if further differentiation is needed treat with oil of cloves. If this is not enough rinse in abs. alc., flood momentarily with 95% alc. and pass back through absolute to toluol. Wash in 2 changes toluol and mount in balsam. This is a difficult method but the results are worth it. (see Bensley, R. R., Am. J. Anat., 1911, 12, 297–388).

**Benzamine Blue 3B,** see **Trypan Blue.**

**Benzene-Azo-$\alpha$-Naphthylamine.** A monoazo dye used by Carter, J. S., J. Exp. Zool., 1933, 65, 159–179 as a vital stain for **Stenostomum.**

**Benzo Blue 3B,** see **Trypan Blue.**

**Benzo New Blue 2B,** see **Dianil Blue 2R.**

**Benzo Sky Blue,** see **Niagara Blue 4B.**

**Benzoin Blue R,** see **Azo Blue.**

**Benzopurpurin 4B** (CI, 448)—cotton red, diamin red, dianil red, Sultan and direct red, all 4B—An acid dis-azo dye no longer used.

**Benzyl Benzoate** is employed in the **Spalteholz Method** of clearing.

**Benzyl Violet.** Conn (p. 132) states that this term relates to a group of violets which are pararosanilins, some acid and some basic, with benzyl substitution in one or more amino groups.

**Berberian's Method.** Berberian (D. A., Arch. Dermat. & Syph., 1937, 36, 1171–1175) has developed a method for staining hair and fungi and for differentiating epithelial cells, blood cells, bacteria and 'mosaic fungi'. Cover small pieces of scales on a slide with 50% aq. glacial acetic acid and dry in an incubator. To defat, clear and dehydrate, cover the preparation with ether 2–3 times, 20–30 sec. each. Then flood twice with absolute acetone, 30–60 min. each. Follow by flooding consecutively with absolute, 95, 70 and 50% alcohol. The stain is Martinotti's (aq. dest., 75 cc.; lithium carbonate, 0.5 gm.; and toluidin blue, 1 gm.). After the stain dissolves, add 20 cc. glycerin and 5 cc. 95% alcohol. Wash the preparation with water and differentiate with 0.5% acetic acid. Dehydration is best carried out in absolute acetone for 2–3 min., then pass through xylol and mount. See **Fungi.**

**Bergamot Oil** is sometimes used for clearing because it will mix with 95% alcohol.

**Berlin Blue** is another name for **Prussian Blue** (a metallic pigment). It is employed for microchemical detection of **Iron.** Kremer, Zeit. f. wiss. mikr., 1938, 54, 429–432 suggests proceeding as

follows: Fix in absolute alcohol. Deparaffinize 10$\mu$ sections. Bleach in 3–5% $H_2O_2$ 3–5 days. Wash carefully in aq. dest. Quickly darken in $(NH_4)_2S$. Transfer to K ferrocyanide and HCl. Iron gives blue color.

Curiously enough when injection of blood vessels is demanded this mineral pigment is usually called for as Berlin blue. Thus the Bensleys (p. 153) give directions for making up Tandler's Berlin blue gelatin. Soak and melt 5 gms. pure gelatin in 100 cc. aq. dest. Add sufficient Berlin blue to give desired color and then 5–6 gms. potassium iodide and a crystal of thymol as a preservative. Inject this mass, which is fluid above 17°C. Fix tissues in 5% formalin which preserves it even through decalcification.

**Best's Carmine.** Grübler's carminum rubrum optimum, or some other good carmine, 2 gm.; potassium carbonate, 1 gm.; potassium chloride, 5 gm.; aq. dest., 60 cc. Boil gently until color darkens, cool and add 20 cc. conc. ammonia. Allow to ripen 24 hrs. This is stock solution. Used to stain **Glycogen.** See Bensley, C. M., Stain Tech., 1939, **14**, 47–52.

**Beyer Brown,** a diazo dye, stains in aq. or alcoholic solution like a good Ehrlich's hematoxylin (H. G. Cannan, J. Roy. Micr. Soc., 1941, **61**, 88–94).

**Bichromate-Chromic-Osmic** mixture, see **Champy's Fixative.**

**Biebrich Scarlet,** water soluble (CI, 280)—croceine scarlet, double scarlet BSF, Ponceau B, scarlet B or EC—An acid dis-azo dye much used in histology. See **Bowie.**

**Biebrich Scarlet and Picro-Anilin Blue,** as a differential stain for connective tissue and muscle (Lillie, R. D., Arch. Path., 1940, **29**, 705). Deparaffinize sections of material fixed in formalin, Zenker's or Orth's fluid. Stain for 5 min. in following: Dissolve 1 gm. hematoxylin in 95% alc. and 4 cc. 29% aq. $FeCl_3$ in 95 cc. aq. dest. + 1 cc. conc. hydrochloric acid. Mix and use while fairly fresh. Wash in tap water. Stain for 4 min. in 0.2 gm. Biebrich scarlet + 100 cc. 1% aq. acetic acid. Rinse again in aq. dest. Stain for 4 min. in 0.1 gm. anilin blue W.S. (C.C.) + 100 cc. sat. aq. picric acid. Wash for 3 min. in 1% aq. acetic acid. Dehydrate in acetone or alcohol. Clear and mount in salicylic acid balsam. Connective tissue, glomerular basement membrane and reticulum, deep blue; muscle and plasma, pink; erythrocytes, scarlet.

**Bielchowsky Silver Methods.** These are designed for the nervous system and consist essentially of formalin fixation, silver impregnation, washing, treating with ammoniacal silver solution, washing and reduction in formalin. Several useful modifications are detailed by Addison (McClung, pp. 463–466). See **Nervous System, Silver Methods.**

**Bile.** This frequently comes in for microscopic examination of centrifuged sediment. Stitt (p. 761) says that one must be on the lookout especially for: (1) Pus cells (neutrophiles), scattered through the specimen and bile stained, which, when occurring in fair numbers, indicate cholecystitis. Unstained pus cells associated with mucus are generally from the mouth. (2) Bile colored epithelial cells and cellular debris suggest chronic cholecystitis. (3) Cholesterin crystals are identifiable as opaque or translucent, flat, rhombic plates or irregular masses. (4) Large amounts of light brown granules or dark black-brown ppt. of calcium bilirubinate are suggestive of gall stones. (5) Tiny gall stones (bile sand) are identifiable by their concentric lamination. Negative findings are not, he is careful to point out, conclusive of absence of lesions.

**Bile Capillaries.** 1. *Hematoxylin staining.* Clara, M., Zeit. f. mikr. Anat. Forsch., 1934, **35**, 1–56 advises treatment of celloidin sections of pieces of liver fixed in **Alcohol Formalin,** formalin—absolute alcohol—acetic acid (20:80:1) and other mixtures by the *Stölzner Holmer* technique and his own method. According to the former, mordant the sections in liquor ferri sesquichlorati (try 10% aq. ferric chloride) 30–45 min. Wash quickly in aq. dest. Stain in ripe 0.5% aq. hematoxylin, 20–30 min. Wash quickly in water. Differentiate in much diluted liquor ferri sesquichlorati. Wash again quickly in water. Blue with dilute aq. lithium carbonate. Wash in spring water (tap water will do). Dehydrate, clear and mount. According to Clara, mordant the sections in equal parts A and B at 40–50°C. for 24 hrs. (A = potassium bichromate, 2.0 gm.; chrome alum, 1 gm., aq. dest., 30 cc. B = ammonium molybdate, 2.5 gm.; chromic acid, 0.25 gm.; aq. dest., 100 cc.) Wash briefly in aq. dest. Stain in **Kultschitzky's Hematoxylin.** Wash in spring water. Dehydrate, clear and mount in balsam. See Clara's illustrations.

2. *Rio Hortega silver carbonate method* adapted by McIndoe, A. H., Arch. Path., 1928, **6**, 598–614. Fix small pieces normal human liver at least 20 days in 10% formalin. Heat gently but do not boil and cool several times thin frozen sections for 20 min. in silver bath until they are uniformly of a golden brown

color. (To make the bath combine 30 cc. 10% aq. silver nitrate and 10 cc. sat. aq. lithium carbonate. Wash ppt. repeatedly with doubly distilled water, decanting washings. Add 100 cc. doubly distilled water to ppt. Dissolve ½–¾ of it by adding ammonia water drop by drop. Filter supernatant fluid into opaque bottle and store in dark where it can be kept 2–4 weeks. For use take 5 cc. of this stock solution and add 5 cc. aq. dest. and 2–3 drops pyridine.) Wash quickly in aq. dest. Place in 20% neutral formalin, 1 min. Fix in 2% aq. sodium thiosulphate, ½–1 min. Wash thoroughly in tap water, 2–3 days adding a little neutral formalin. Dehydrate in 95% and abs. alc., clear in carbol-xylol and mount in balsam. Canaliculi, black.

**Bile Components** in hepatic cells. Place small pieces of liver in 3% aq. barium chloride for 6 hours; fix 18 hours in 10% formalin; dehydrate rapidly in alcohol, clear in benzol and embed in paraffin. The bile components, precipitated by barium chloride, can be stained with acid dyes especially the acid fuchsin in Mallory's connective tissue stain (Forsgren, E., J. Morph., 1929, **47**, 519–529).

**Bile Pigments.** Histochemical reaction. Fix in 10% formalin or in alcohol. Prolonged fixation is contraindicated. Fix paraffin sections to slides with egg albumen. Deparaffinize and immerse in 2 or 3 parts Lugol's solution and 1 part tincture of iodine, 6–12 hrs. Wash in aq. dest. and cover with sodium hyposulphite (5% aq.) 15–30 sec. until decolorized. Wash in aq. dest. and stain with alum carmine 1–3 hrs. Wash in aq. dest., dehydrate in acetone, clear in xylol and mount in balsam. Bile pigment granules emerald green (Stein, J., C. R. Soc. de Biol., 1935, **120**, 1136–1138). See **Gmelin's Test.**

**Bilharzial Cercariae.** For intra-vitam staining examine in serum plus a little neutral red. For permanent preparations fix in hot lactophenol (equal parts lactic acid, carbolic acid, glycerin and aq. dest.). Stain with alcoholic borax-carmine. Mount in following: dissolve by boiling gum tragacanth 3 parts and gum acacia 1 part in aq. dest. 100 parts. Add equal parts lactophenol and use filtrate. (Marshall, A., Lab. J., 1937, **7**, 565–569).

**Bilirubin,** a reddish bile pigment which is isomeric or identical with **Hematoidin** and which by oxidation can be converted into the green **Biliverdin,** see **Bile Pigments.**

**Biliverdin,** a green bile pigment produced by oxidation of **Bilirubin.** See **Bile Pigments.**

**Bindschedler's Green** (CI, 819). A basic indamin dye easily reduced to a substituted diphenylamine. See use as a **Redox** dye in study of metabolism of tumor tissue (Elliott, K. A. C. and Baker, Z., Biochem. J., 1935, **29** (2), 2396–2404).

**Bird's Eye Inclusions.** Some of these bodies, and the so-called **Plimmer's Bodies,** seen in cancer cells are apparently greatly enlarged **Centrosomes.** Methods and results are given by LeCount, E. R., J. Med. Res., 1902, 7 (N.S. 2), 383–393.

**Bismark Brown Y** (CI, 331)—basic brown, G, GX, or GXP, Excelsior brown, leather brown, Manchester brown, phenylene brown, Vesuvin—A mixture of basic dis-azo dyes of different shades. Quite widely employed, see Blaydes, G. W., Stain Techn., 1939, **14**, 105–110 for use with plant tissue.

**Bismiocymol** (see Pappenheimer, A. M. and Maechling, E. H., Am. J. Path., 1934, **10**, 577–588.

**Bismuth.** Microchemical detection of:

1. Method of Christeller-Komaya. Make frozen sections of formalin fixed tissues. A = quinine sulphate, 1 gm.; aq. dest., 50 cc.; nitric acid, 10 drops. B = potassium iodide, 2 gm., aq. dest., 50 cc. Immediately before use mix equal parts A and B and add 2 drops nitric acid, C.P. After treating sections with this for 1 min. wash very quickly in 10 cc. aq. dest. + 2 drops nitric acid. Mount section on slide. Dry, counterstain with gentian violet. Bismuth appears as dark brown grains (Lison, p. 98). *See* Komaya, G., Arch. f. Dermat. u. Syph., 1925, **149**, 277–291 (good colored figures) and Califano, L., Zeit. f. Krebsf., 1927–28, **26**, 183–190.

2. Another modification of the Komaya method is given by Castel, P., Arch. Soc. d. Sci. Med. et. biol. de Montpellier, 1934–35, **16**, 453–456 as follows: Dissolve 1 gm. quinine sulphate in 50 cc. aq. dest. with aid of a few drops of sulphuric acid. Dissolve 2 gm. potassium iodide in 50 cc. aq. dest. Mix, apply to section, gives red ppt. of salts of bismuth in form of iodobismuthate of quinine or double iodide of bismuth and quinine. See Pappenheimer and Maechling's (Am. J. Path., 1934, **10**, 577–588) study of nuclear inclusions in the kidney.

**Bismutose,** a compound of bismuth and albumen which on application becomes concentrated in the area of the Golgi apparatus (Kredowsky, Zeit. f. Zellf., 1931, **13**, 1).

**Blastomycosis.** The differentiation of *Zymonema* (*Blastomyces*) *dermatitidis*, the cause of blastomycosis, from *Crypto-*

*coccus hominis*, the cause of cryptococcosis or torulosis, is best accomplished by wet India ink technique of Weidman, F. D. and Freeman, W., J.A.M.A., 1924, **83**, 1163. Stir suspected material in a drop of india ink, place on a clean slide and cover. Use a small drop so as to form a thin film. Work rapidly before the ink dries out. In blastomycosis the wall of the organism is thick and presents a double-contoured appearance. *Cryptococcus hominis* is surrounded by a thick mucoid capsule which, against a dark background, shows up as a clear halo surrounding the fungus. Spinal fluid usually dilutes the ink making a lighter background. See **Fungi.**

**Blood.** Microscopically considered blood is the field of the hematologist (see Downey's Hematology, N. Y., Hoeber, 1938 in 4 volumes). Any conception of the formed elements of the blood is artificial and inadequate unless it is based upon knowledge of their appearance and behavior *in vivo*. To examine circulating blood in the web of a frog's foot is helpful but it is better to use mammals. In the latter, the methods devised by Covell and O'Leary for study of the living **Pancreas** are recommended for blood cells also. Probably the best technique is that of **Sandison** for direct examination of contents of small blood vessels and capillaries in transparent chambers inserted into rabbits' ears. Living blood cells can be observed *in vitro* at high magnification in **Tissue Cultures**; but, of course, circulation is lacking.

When blood cells are removed from the body and mounted on slides in approximately isotonic media, they can be studied for a short time before they become seriously injured and die. Examination in the dark field and after **Supravital Staining** may be helpful. It is important in interpreting the results to remember that the conditions are very abnormal, that the cells are often more flattened than *in vivo*, and that the actual speed of movement is not that seen, but is that observed divided by the magnification because the distance travelled per unit of time naturally appears greater than it actually is. The motion picture technique has great potentialities.

Examination is usually limited to fixed and stained **Blood Smears** but valuable data can also be secured from sections. Normal values for blood cells during first year of life (Merritt, K. K., 1933, **46**, 990–1010). For details, see **Blood Protein** (coagulated), **Bone Marrow, Chylomicrons, Erythrocytes, Erythrocyte Counts, Fibrin, Hematoidin, Hematoporphyrin, Hemofuscin, Hemoglobin, Hemosiderin, Leucocytes, Leucocyte Counts, Lymphocytes, Monocytes, Platelets, Parhemoglobin, Reticulocytes, Sulfmethemoglobin.**

**Blood Cell Volume.** Dry Evans Blue (Merck) at 100°C. Dissolve 400–800 mg. in 1 liter aq. dest. Put 0.5–1 cc. in tube 3–4 cc. capacity and evaporate to dryness at 70°C. Collect blood to contain 2.0–2.5 units heparin or 0.2% ammonium oxalate. Centrifuge and transfer 1 cc. plasma to tube containing dye. Remove 0.1 cc. dyed plasma to 9.9 cc. saline in photoelectric colorimeter tube. Make blank without plasma. Compare in Evelyn or Klet-Summerson colorimeter using filter to pass only light of about 620 mμ. Calculate as directed for the colorimeter (Shohl, A. T. and Hunter, T. H., J. Lab. & Clin. Med., 1941, **26**, 1829–1837).

**Blood Protein.** Coagulated blood protein within the vascular lumina of stained sections of fixed tissues is an artifact in the sense that its appearance has been greatly modified by the technique. It is sometimes made up of particles of quite uniform size and has been mistaken for masses of microorganisms; but it does not exhibit both acidophilic and basophilic staining reactions suggestive of cytoplasmic and nuclear components.

**Blood Smears.** These should be made on slides rather than on cover glasses for several reasons. A larger film of blood is thereby provided for examination. Smears on slides are easier to make and to handle. They can be studied without covering them whereas a smear on a cover glass cannot be moved about on the stage of the microscope unless it is mounted smear side down on a slide. The colors are often more permanent in smears on slides which are not covered with cover glasses. A good way is to spread a thin film of immersion oil over them. This dries much more quickly than balsam or any other medium under a cover glass.

Slides of good quality with ground edges and scrupulously clean are necessary (**Cleaning Glassware**). A finger tip or ear lobule is first cleaned with 95% alcohol. As soon as the surface has dried a small puncture is made with a previously sterilized needle. Special needles with lance shaped cutting ends are better than ordinary pointed ones. A small droplet of blood should appear on slight pressure. The first is wiped away with sterile gauze and the second and following ones are used. Unless the blood is very strongly pressed out,

the differential count of white cells will not be affected. Some advise holding the fingers in hot water before hand to produce a temporary hyperemia in them but this is seldom advisable. A droplet of size sufficient to produce a smear of the desired thickness (determined by trials) should be touched to the surface of a slide about 3 cm. from one end conveniently placed on a table. Immediately the end of a second slide, with its edge squarely across the first slide is brought in contact with the blood on the remote side of the drop from the nearest end of the first slide. The blood spreads quickly along this edge toward the sides of the slide on the table which is steadied with the left hand. The end edge of the second slide is slowly but steadily pushed the length of the first slide and the blood is drawn out in a thin layer after it. The angle of inclination of the second to the first slide determines the thickness of the smear. It is well to make the first smear at an angle of about 45 degrees; increase it for a thicker smear and decrease it for a thinner one. In the making of smears it is important to have plenty of elbow room. To make good smears is a fine art and a credit to the individual.

Blood smears, whether simply dried by waving in air or thereafter fixed by gently heating, retain their staining properties for a few days but they should be colored without undue delay. It is both wasteful and undesirable to cover the whole slide with stain. Part of the slide will have to be used for record written with a diamond pencil. Therefore draw two lines across the slide near each end with a wax pencil or a piece of paraffin. The stain added with a dropper will cover only the intervening part. For stains see **Giemsa, Wright, Ehrlich, Oxidase, Peroxidase** and **Gordon's Silver Method.**

**Blood Vessels.** These comprise structures of different sorts, existing in a wide variety of environments, which can be investigated from many angles. Consequently to present examples of available techniques under the expected headings involves a lot of mind-reading. The blood vessels of the **Skin** are of course the most accessible. Detailed methods for their direct and indirect study are presented by Sir Thomas Lewis (The Blood Vessels of the Skin and their Responses. London: Shaw & Sons, 322 pp.).

But to microscopically examine all the blood vessels of any particular organ is not possible in the living state because of lack of accessibility, thickness and other mechanical obstacles. Resort is therefore made to various devices for viewing the vessels by themselves unobscured by surrounding tissue. The unwanted tissue is removed by corrosion when the vessels are demonstrated by **Neoprene** injection. It is simply passed over when x-ray photographs are examined after the vascular injection of radiopaque substances like **Bismuth Sulphate** and **Diotrast.** It is rendered transparent when the vessels are filled with easily visualized materials such as **Carmine** or **Berlin Blue,** or is relatively colorless after their walls are selectively stained by **Janus Green, Silver Citrate** or **Silver Chloride Dichlorfluoresceinate.**

Though the larger blood vessels are too thick and cumbersome for microscopic study *in vivo*, this is not so with the smaller ones. Indeed excellent moving pictures can be made of them. A film entitled "Control of Small Blood Vessels" by G. P. Fulton and P. R. Lutz of Boston University is very helpful. The supravital method of studying **Nerve Endings** with methylene blue must be combined with careful dissections (Woollard, H. H., Heart, 1926, **13,** 319–336) in order to gain an impression of the innervation of blood vessels. See **Arteries, Arterioles, Capillaries, Sinusoids, Venous Sinuses, Venules, Arteriovenous Anastomoses, Veins, Vasa Vasorum, Valves, Perfusion.** See **Quartz Rod Technique.**

**Bodian Method.** For staining nerve fibers in paraffin sections (Bodian, D., Anat. Rec., 1937, **69,** 153–162; MacFarland, W. E. and Davenport, H. A., Stain Tech., 1941, **16,** 53–58). The following details of this very useful technique have been supplied by Dr. J. L. O'Leary. Fix by vascular perfusion, with 80% alcohol containing 5% formol and 5% acetic acid, or by immersion in 10% formalin or Bouin's fluid. For *boutons terminaux*, perfuse tissue with 10% chloral hydrate and extract tissue with alcohol for several weeks. Run paraffin sections (15$\mu$ or less) to aq. dest. Place in 1% Protargol (Winthrop Chemical Co.) with 4–6 grams. of metallic copper per 100 cc. (This can be used only once.) Wash in *redistilled* water 1 change. Transfer for 10 min. to: hydrochinone, 1 gm.; sodium sulfite, 5 gm.; aq. dest., 100 cc. Wash in *redistilled* water 1 change. Tone in 1% gold chloride with 3 drops of glacial acetic acid per 100 cc., 5–10 min. Wash in *redistilled* water 1 change. If sections do not have a light purple color place in 2% oxalic acid until the entire section has the slightest blue or purplish tinge.

Pour off as soon as tissue gets slightly blue. Remove residual silver salts in 5% sodium thiosulfate 5–10 min. Wash, dehydrate, clear and mount. *Note*: the Coplin jars used must be cleaned in **Cleaning Fluid.**

**Boedeker's Method,** see **Enamel** matrix.

**Bollinger Bodies,** see **Borrel Bodies.**

**Bone.** A good account of methods is provided by Shipley (McClung, pp. 344–352). Examination *without decalcification* involves the cutting and grinding of thin sections. The instruments used by dentists for the making of sections of undecalcified teeth are of the greatest service and should be purchased or borrowed. If they are not available **Grieves'** method for dental tissues is suggested. In order to determine the structure of bone with *organic material removed*, Shipley advises cutting away all soft parts after which the bone may or may not be split. Place in tap water, or in a 2% aq. gelatin, to which a loop full of culture of *B. coli* has been added. After 5–6 days wash in running water 24–48 hrs. in a stink cupboard. This will dissolve and wash away all organic material. Sterilize the bone by boiling or immersion in alcohol. Saw into sections, grind these to the necessary thinness and polish. Dehydrate in ether. Dry thoroughly and mount in balsam. Routine examination includes some method of fixation, decalcification and staining. Hematoxylin and eosin are recommended, likewise phosphomolybdic acid hematoxylin and Mallory's connective tissue stain.

For different structural components special techniques are required. *Bone corpuscles* may be isolated by putting a thin section of bone in concentrated nitric acid for a few hours to a day. Then place the section on a slide, cover. Pressure on the cover glass will squeeze out ellipsoidal bone cells with their processes (Shipley). *Bone lamellae* can be peeled off easily when decalcified bone has been allowed to simmer in water for several hours (Shipley). *Lacunae and canaliculi.* The easiest method is to impregnate sections of ground bone with 0.75% aq. silver nitrate for 24 hours. Wash, polish the sections on a fine hone to remove precipitated silver, dehydrate in alcohol, clear in xylol and mount in balsam. The lacunae and canaliculi appear black in a yellowish brown background. To impregnate thin sections with acid fuchsin, dry them after extraction with alcohol. Place them in watch glasses in a 20% aq. sol. of acid fuchsin in a desiccator connected with a suction apparatus. Extract air for about an hour and close the dessicator. After 24 hrs. the solution will have dried. Rub off ppt. on a hone, pass through xylol and mount in damar or balsam (Shipley). *Linings of lacunae and canaliculi.* (Schmorl's method modified by Shipley). Employ a fixative not containing mercury. Decalcify in Müller's fluid, wash in running water, embed in celloidin and section not over 10 microns. Stain in thionin solution alkalinised by 2 drops ammonia. Transfer with glass needle to sat. aq. phosphotungstic or phosphomolybdic acid. Leave until blue, gray or green. Place in water until sky-blue. Ammonium hydroxide 1 cc. and aq. dest. 10 cc., 3–5 min. Several changes 90% alcohol. 95% alc. Clear in carbol-xylol and mount in damar (or balsam). This method is suggested for bones of children. *Processes of young osteoblasts in growing bone.* Shipley suggests following treatment of slices of bone of a rickety animal. 4% aq. citric acid 20–30 min. in the dark. Rinse in aq. dest. 1% aq. gold chloride in the dark, 20–30 min. 3% formic acid in the dark, 48 hrs. Rinse in aq. dest. and preserve in pure glycerin. Make frozen sections, mount in glycerin and ring with damar, balsam, paraffin or cement. Keep specimens in dark when not in use.

To determine relative *age of deposition* the following method has proved useful in senile osteoporosis. Saw sections of bone not more than 0.5 cm. thick and fix in 4% formalin 2–4 days. Decalcify in 6% isotonic formalin, 40 cc., 85% formic acid, 60 cc., and sodium citrate, 5 gm. changing every second day for, say, a week, that is until they become flexible and can be penetrated by a fine needle. Embed in celloidin (slow method). Prepare stain by dissolving 30 gm. potassium alum in 1 liter hot water and by adding 1.5 gm. hematoxylin crystals. Cool and add 1 gm. chloral hydrate. Ripening in sunlight to rich dark color is hastened by addition of crystal of potassium hydroxide. Stain celloidin sections about 2 days checking by microscopic examination until some areas are definite violet azur, others lighter or colorless. Wash in tap water 24 hrs. Stain in 100 cc. aq. dest. + 2–3 drops 1% aq. eosin 1–2 days (uncolored areas become dark rose color). Dehydrate, clear in xylol and mount in balsam. Old bone azur; new bone bright rose (Belloni, L., Arch. Ital. Anat. e Istol. Path., 1939, **10,** 622). See **Madder** staining of new bone, **Alizarin Red S** staining of dentine, various tests for **Calcium,** and **Ossification, Line Test** for vitamin D potency.

Polarized light is excellent for the demonstration of bone camellae.

**Bone Marrow.** Microscopic examination of bone marrow *in vivo* has not been achieved because of the obvious technical difficulties involved. The best that can be done is to study still living cells removed from bone marrow unstained or supravitally stained. The methods are essentially the same as for blood. From humans samples can be obtained by sternal puncture (Young, R. H. and Osgood, E. E., Arch. Int. Med., 1935, **55**, 186–203, and many others). Primitive cells of the erythrocytic and leucocytic series can only be identified when hemoglobin and specific granules respectively appear within them. Microchemical tests for **Hemoglobin** should be more used. For the granules the methods of **Giemsa, Wright, Ehrlich** and others are the best available. Special techniques have been described for **Megakaryocytes** particularly in relation to platelet formation. To demonstrate the vascular pattern special methods are required (Doan, C. A., Johns Hopkins Hosp. Bull., 1922, **33**, 222–226). To reveal the nerve supply is particularly difficult. Glaser (W., Ztsch. f. Anat. u. Entw., 1928, **87**, 741–745) has described a fine network accompanying the vessels but Doan and Langworthy (Downey, p. 1852) were less successful. Sternal bone marrow during first week of life (Shapiro, L. M., and Bessen, F. A., Am. J. Med. Sci., 1941, **202**, 341–354). Bone marrow of normal adults (Plum, C. M., Acta Med. Scand., 1941, **107**, 11–52. See chapters by Sabin and Miller and by Doan in Downey's Handbook of Hematology, New York, Hoeber, 1938, **3**, 1791–1961 for details.

**Borax Carmine** (Grenacher). Make con. sol. of carmine in borax (2–3% carmine in 4% aq. borax) by boiling for 30 min. Allow to stand 2–3 days with occasional stirring. Dilute with equal volume 70% alc., again allow to stand and filter. Much used for staining tissues in bulk. They are colored for days if necessary, transferred directly to acid alc. (70% alc. 100 cc., hydrochloric acid 4 drops) in which they assume a bright red transparent appearance. Then wash in alcohol, mount as whole specimens or imbed in paraffin and cut sections. Borax carmine can also be employed to stain sections (Lee, p. 146).

**Borax Ferricyanide,** see **Weigert's.**

**Bordeaux,** see **Amaranth.**

**Bordeaux Red** (CI, 88)—acid Bordeaux, archelline 2B, azo-Bordeaux, cerasin R, fast red B, BN or P—An acid mono-azo dye very widely employed in histology.

**Bordeaux SF,** see **Amaranth.**

**Borrel Bodies** (Bollinger bodies) in fowl pox. References to earlier staining methods and directions for applying the microincineration technique with figures showing the comparative results are given by Danks, W. B. C., Am. J. Path., 1932, **8**, 711–716. See microincineration of Molluscum bodies (Van Rooyen, C. E., J. Path. & Bact., 1939, **49**, 345–349).

**Borrelia Vincenti,** see **Vincent's Angina.**

**Borrel's Stain.** Fix in osmic acid, 2 gm.; platinum chloride, 2 gms.; chromic acid, 3 gm.; glacial acetic acid, 20 cc. and aq. dest., 350 cc. for 24 hrs. Wash in running water several hours. Dehydrate, clear, imbed and section. Stain sections in 1% aq. magenta 1 hr. Then in sat. aq. indigo-carmine, 2 parts and sat. aq. picric acid, 1 part. Wash in alc., dehydrate, clear and mount. The above has been partly taken from Lee's Vade Mecum, p. 433. Other more convenient fixatives will do equally well. The stain has been used for the *Borrel bodies* in fowl pox.

**Botanical Technique.** Many of the methods used in animal histology are applicable also in plant histology and vice versa. Details are given in a chapter by W. R. Taylor in McClung, p. 155–245. See **Plants.**

**Bouin's Fluid.** Sat. aq. picric acid, 75 cc.; formalin, 25 cc.; acetic acid, 5 cc. For mammalian tissues fix 24 hrs., wash in water, dehydrate and embed in the usual way. This is the most generally useful of all fixatives containing picric acid. Almost any stain can be used after it. The picric acid need not be altogether washed out because it serves as a desirable mordant. Giemsa's stain gives good coloration of protozoan parasites after fixation in Bouin's fluid (Cowdry, E. V. and Danks, W. B. C., Parasitology, 1933, **25**, 1–63). The use of this fixative is specified under **Argentaffine Reaction, Bodian's Method, Elementary Bodies, Foot's Method, Gold, Johnson's Neutral Red Method, Laidlaw's Method, Liebermann-Burchardt Reaction, Masson's Trichrome, Purkinje Cells, Tapeworm Proglottids,** etc. It is a fixative rapidly gaining in popularity and there are naturally many modifications. The application of Davenport's silver technique to Bouin fixed tissues is described by Foley, J. O., Stain Techn., 1938, **13**, 5–8.

The cytological action of Bouin's fluid has been investigated at the University of Pennsylvania. Three formulae are particularly recommended by McClung and Allen (McClung, p. 561). (1) *Allen's fluid*: sat. aq. picric acid, 75 cc.; formalin C.P., 15 cc.; glacial acetic acid, 10 cc.; urea, 1 gm. (2) The same plus

1 gm. chromic acid. (3) The original formula plus 2 gms. urea and 1.5 gms. chromic acid. For details regarding use in study of cell division, shrinkage, etc. see Allen, Ezra, Anat. Rec., 1916, **10,** 565–589.

**Boutons Terminaux.** For this special type of nerve ending the methods given under **Nerve Endings** are useful, particularly that of **Bodian.** These terminal buttons or swellings can be visualized and their behavior determined in living tadpoles by techniques introduced by Speidel, C. C., J. Comp. Neurol., 1942, **76,** 57–73. Several special methods for their demonstration in fixed tissues are recommended by Gibson (McClung, pp. 481–488).

**Bowie's Ethyl Violet-Biebrich Scarlet** stain for pepsinogen granules (Bowie, D. J., Anat. Rec., 1935–36, **64,** 357–367). Dissolve 1 gm. Biebrich scarlet in 250 cc. aq. dest. and 2 gms. ethyl violet in 500 cc. Filter the former through a rapid filter paper into a beaker and then the latter into the same beaker. The end point of neutralization is when a little of the mixture placed on filter paper does not show any scarlet color. Collect the ppt. of neutral dye by filtering and dry it. To make stock solution dissolve 0.2 gm. in 20 cc. 95% alcohol. To make staining solution add 1–5 drops to 50 cc. of 20% alcohol. Stain paraffin sections of Regaud fixed gastric mucosa in this for 24 hrs. Wipe dry around edges and blot with smooth filter paper. Differentiate by covering section with equal parts clove oil and xylol. This takes about 30 min. and should be observed under microscope. Pass through several changes of xylol, rinse in benzol and mount in benzol balsam. Pepsinogen of pepsin-forming cells, violet; and parietal cells, red. Bowie also makes a crystal violet-orange G stain which does not differ materially from Bensley's **Neutral Gentian.**

**Brandt's** glycerin jelly. Melted gelatin, 1 part; glycerin 1½ parts plus few drops carbolic acid to serve as a preservative. See Kaiser's glycerin jelly under glycerin.

**Brazilin** (CI, 1243) is a substance produced from red wood of Brazil. Its formula is like that of hematoxylin minus 1 hydroxyl group and in its use, as well as its origin, it resembles hematoxylin. Ripening may be required for both. Thus we have an iron brazilin method (Hickson, S. J., Quart. J. Micr. Sci., 1901, **44,** 469–471) and **O'Leary's Brazilin** for myelin sheaths. See also **Brazilin-Wasserblau** technique of Bensley.

**Brazilin-Wasserblau** for secretion antecedents of thyroid gland (Bensley, R. R., Am. J. Anat., 1916, **19,** 37–54) as described later by the Bensleys (p. 80) is: To make up the Brazilin stain dissolve 0.05 gm. in a little aq. dest. with aid of heat and add this to 100 cc. 1% aq. phosphotungstic acid. Ripen by addition of 2 drops hydrogen peroxide. Solution should not be employed after 3 days. Run paraffin sections of formalin-Zenker fixed thyroids down to aq. dest., mordant briefly in a fresh aq. ammonium stannic chloride, and stain in above solution 1 or more hrs. Wash in water and treat for 1–5 min. with aq. dest., 100 cc. + 1.0 gm. phosphomolybdic acid and 0.2 gm. Wasserblau (anilin blue). Wash quickly in water, dehydrate in absolute alcohol, clear and mount. See colors in R. R. Bensley's plate. Nuclear chromatin, red; secretion antecedents in pale blue droplets; mitochondria, reddish-purple; connective tissue, blue; erythrocytes, orange-red; etc.

**Breast,** see **Mammary Glands.**

**Brilliant Blue C,** see **Brilliant Cresyl Blue.**

**Brilliant Congo R,** see **Vital Red.**

**Brilliant Congo Red R,** see **Vital Red.**

**Brilliant Cresyl Blue** (CI, 877)—brilliant blue C, cresyl blue 2RN or BBS—Commission Certified. This basic oxazin dye is used for making **Platelet Counts** and for many other purposes.

**Brilliant Dianil Red R,** see **Vital Red.**

**Brilliant Fat Scarlet B,** see **Sudan R.**

**Brilliant Green** (CI, 662)—diamond green, ethyl green, malachite green G, solid green JJO—Commission Certified. This di-amino tri-phenyl methane dye is used to color culture media.

**Brilliant Pink B,** see **Rhodamine B.**

**Brilliant Ponceau G,** see **Ponceau 2R.**

**Brilliant Purpurin R** (CI, 454). An acid dis-azo dye. Conn (p. 62) says that this is the dye which Gutstein, M., Zeit. f. Ges. Exp. Med., 1932, **82,** 479–524 called "brilliant purpur R" and used as a vital stain for yeasts.

**Brilliant Vital Red.** Use in determination of plasma volume is justified, since the dye is not taken into the erythrocytes (Gregersen, M. I., and Schiro, H., Am. J. Physiol., 1938, **121,** 284–292). See **Vital Red.**

**Bromine.** According to Lison (p. 110) bromine has not been investigated histochemically in animal tissues. For its detection in plants consult Mangenot, H. G., Bull. d'Hist. Appl., 1927, **4,** 52–71.

**Brown Salt R,** see **Chrysoidin Y.**

**Brownian Movement.** Calculation of cytoplasmic viscosity through measurement of displacement of particles in Brownian movement gives results not very differ-

ent from determinations by the centrifugation method (Danielli in Bourne, p. 31).

**Buffalo Garnet R,** see **Erie Garnet B.**

**Buffers.** For many purposes it is essential to use solutions buffered at a certain pH. Details concerning numerous buffers are given by Clark, W. M., The Determination of Hydrogen Ions. Baltimore: Williams & Wilkins, 1928, 717 pp. French, R. W., Stain Techn., 1930, **5,** 87–90 (see correction, 1932, **7,** 107–108) recommends **Sorensen's** phosphate mixtures and **Palitzsch's** borax-boric acid mixtures each over certain ranges of pH. He emphasizes the fact that the addition of buffer salts is known to have a decided influence in some cases on the behavior of the dyes irrespective of pH. See also **Clark** and **Lubs Buffers.**

Petrunkevitch, A., Anat. Rec., 1937, **68,** 267–280 explains that aqueous solutions of stains at certain pH's are more selective than alcoholic ones and that the greatest differentiation is obtained with the former ones with pH suitably adjusted by addition of HCl or NaOH. Next in desirability come stains dissolved in acetate, phosphate and borate buffers. Citrate buffers are in his experience less suitable because a more diffuse staining results while phthalate buffers should not be used. He gives specific directions for the preparation of solutions at pH of maximum staining of acid fuchsin, aniline blue, aurantia, benzoazurine, eosin Y, light green, metanil yellow, methylene blue, orange G, toluidin blue, Wrights stain and eosin methylene blue.

For safranin O, see Sawyer, C. H., Stain Techn., 1940, **15,** 3–7 and for hematoxylin, malachite green and eosin Y, Craig, R. and Wilson, C., *ibid,* 1941, **16,** 99–109. Levine, N. C., *ibid,* 1940, **15,** 91–112 contributes useful data on buffered stains in relation to isoelectric point of cell components. Obviously the maximum intensity of staining depends not only on pH but also on properties of substances stained and their treatment from beginning to end of the technique. Lillie, R. D., Stain Techn., 1941, **16,** 1–6 employed **McIlvaine** citric buffers in order to improve Romanowsky staining (see **Toluidine Blue Phloxinate**) after various fixatives. See **McJunkin-Haden Buffer.** For buffering in connection with silver impregnation see Davenport, H. A., McArthur, J. and Bruesch, S. R., Stain Techn., 1939, **14,** 21–26; Silver, M. L., Anat. Rec., 1942, **32,** 507–529. When accuracy is essential check the actual pH of the solution to which buffers have been added by the glass electrode method which anyone can learn to use in a few hours and which gives the answer very quickly. See **Hydrogen Ion Indicators.**

**Bundle of His,** see Todd, T. W., Cowdry's Special Cytology, 1932, **2,** 1173–1210.

**Butter Fat,** reactions in tissue to fat stains after various fixations (Black, C. E., J. Lab. & Clin. Med., 1937–38, **23,** 1027–1036).

**Butyl Alcohol,** see **n-Butyl** and **Tertiary Butyl.**

**Buzaglo's Connective Tissue Stain.** (Buzaglo, J. H., Bull. d'Hist. Appl., 1934, **11,** 40–43). This method is intended to replace that of Van Gieson. Solutions required: (1) *Gallocyanin* (Hollborn, 2264). Boil 0.1 gm. in 100 cc. 5% aq. chrome alum for 10 min. After cooling make up to 100 cc. with aq. dest., filter and add a little formalin to filtrate. (2) *Orcein* (Hollborn, 2466). Dissolve 1 gm. in 100 cc. acid alcohol (70% alcohol, 100 cc. + 1 cc. hydrochloric acid standard). (3) *Acid alizarin blue* (Hollborn, 2559). Boil for 10 min. 5 gm. in 100 cc. 10% aq. aluminum sulphate. After cooling make up to 100 cc., filter and add formalin. (4) *Alizarine-viridine* (Hollborn, 2035). Dissolve 0.2 gm. in 100 cc. aq. dest. acidulated to pH 5.8 with hydrochloric acid. He advises fixation in formalin, **Maximow's** fluid, **Susa** or Hoffker (of which he does not give composition). Pass sections (presumably paraffin) down to aq. dest. Stain nuclei in gallocyanin as deeply as possible 5 times, 24 hrs. Rinse twice in aq. dest. Stain elastic fibers in orcein, then aq. dest., 3 times, 5 min. Stain muscle in acid alizarin blue, 7 min., aq. dest. twice. Differentiate in 5% aq. phosphomolybdic acid 25–30 min., aq. dest. twice. Stain collagen in alizarine viridine 7 min. Blot with 4 layers filter paper. 95% alc. 96% alc. Carbol-xylol, 2 changes xylol. Balsam. Nuclei, dark blue; elastic fibers, red brown; muscle and epithelium, pale blue violet; collagen, mucus, cartilage, shades of green; myelin sheaths, rose; axis cylinders, dark blue; erythrocytes, red brown.

**Cadmium.** The *chloride* is employed in fixation of Golgi apparatus prior to silver impregnation (Aoyama, F., Zeit. wiss. mikr., 1929, **46,** 489–491). See comment by Baker (Bourne, p. 19) on this and use by Ciaccio of cadmium *nitrate* to render phospho- and galactolipines less soluble. Bourne (p. 106) refers to Joyet-Lavergne's claim that cadmium lactate reacts with glutathione in the cell producing a cadmium glutathione compound which is microscopically visible.

**Cajal's.** 1. Brom-formol-silver method for neuroglia. Details supplied by Dr. J. L. O'Leary. Fix small fresh pieces, 3–15 days, in: aq. dest., 85 cc.; formalin, 15 cc.; ammonium bromide, 2 gm. Cut 25$\mu$ frozen sections and return to: aq. dest., 50 cc.; formalin, 6 cc.; ammonium bromide, 3 gm. for 4–6 hrs. at 30–38°C. or for 8–10 hrs. at room temperature. Wash for a few seconds in aq. dest. Place in the following fluid in a porcelain dish and heat over the flame: aq. dest., 10–15 cc.; ammoniacal silver oxide, 5 cc.; pyridine C.P., 4–5 drops. (To prepare silver oxide solution: Take 10 cc. 10% silver nitrate, add 12 drops 40% NaOH. Collect the ppt., wash 5–6 times with aq. dest., then add ppt. to a beaker containing 60–70 cc. aq. dest. Redissolve with least quantity of ammonia necessary. If too much ammonia is added, results are bad.) Remove when sections have reached a tobacco brown color. Wash through 2 changes aq. dest. not more than 5 sec. in all. Reduce in 5% formalin for 2–3 min. Tone with 0.2% aq. gold chloride and fix in 5% aq. sodium hyposulfite. After washing carry to 95% alcohol, carbol-xylol, xylol balsam. See **Microglia** and **Oligodendroglia.**

2. Chloral hydrate method as described by Willard, D. M., Quart. J. Micr. Sci., 1935–36, 78, 475–485 for innervation of adrenal. Fix for 24 hrs. in: chloral hydrate, 2.5 gm.; 95% alcohol, 40 cc.; aq. dest., 40 cc.; pyridine, 20 cc. Wash in aq. dest. until smell of pyridine disappears. 97% alcohol, 24 hrs. Wash again in aq. dest. and transfer to 2.5% aq. silver nitrate at 37°C. for 9–12 days (longer times better for nerve cells). Wash for 1 min. in aq. dest. Reduce for 12–24 hrs. in: hydroquinone, 1 gm.; neutral formol, 10 cc.; aq. dest., 90 cc. Dehydrate rapidly, imbed in paraffin and cut 15–30$\mu$ sections. Nerve fibers, black; background, yellow.

**Cajal Silver Methods.** These depend mainly on silver impregnations reduced by photographic developers such as hydroquinone. They have all been very greatly improved by a preliminary fixation and in other ways and have played a leading rôle in neurology. See **Ranson** pyridine method and other modifications given by Addison (McClung, pp. 452–463). Many techniques spring from a combination of Cajal and Bielchowsky methods.

**Calcareous** deposits. Vital staining with **Alizarin Red S** (Ham, A. W., Arch. Path., 1932, **14**, 613–626).

**Calcium.** There is no absolutely specific microchemical test for calcium in sections. A critical account by Cameron (G. R., J. Path. and Bact., 1930, **33**, 929–955) affords instructive reading.

1. *von Kossa test.* Sections are transferred from aq. dest. to 10% silver nitrate and exposed to bright light for 30 min. or more. Wash carefully in aq. dest. Mount in glycerin, or dehydrate clear and mount in balsam. Inorganic material in most cases calcium phosphate or carbonate is deep black.

2. *Alizarin.* Sections from aq. dest. are stained in 1% aqueous alizarin S (sodium alizarin sulphonate) or in 1% alcohol tetra-hydroscy-anthraquinon (or anthrapurpurin) for an hr. or more. They are then differentiated in 1 part concentrated ammonia and 9 parts absolute alcohol. This is followed by rapid washing in acid alcohol (hydrochloric acid 5 cc., 95% alcohol 95 cc.). It may be desirable to alternate alkali and acid alcohols 2 or 3 times. Wash thoroughly in aq. dest.; dehydrate clear and mount. The alizarin forms a fast compound with earthy salts especially calcium more easily in young than in old bones. Substances may exist in the tissues that inhibit the combination (see **Bone, Madder** staining).

3. *Hematoxylin.* This is not, as is generally supposed, a stain for calcium though it may color calcium as well as other materials when mordanted with chromium salts or alum. According to Cameron, in bone, "staining with hematoxylin is dependent on the essential ground substance and the presence of certain heavy metals especially iron chromium and aluminum; it has no direct relation to calcium salts." He thinks that areas of pathological calcification which stain deeply with alum hematoxylin do so because of the presence of free iron.

4. *Fluorescence x-radiation.* Used for thin sections of undecalcified bone. It is not feasible to magnify much but the method is said to be almost specific for calcium (Dershem, E., Proc. Nat. Acad. Sci., 1939, **25**, 6–10).

5. Cretin, A., Bull. d'Hist. Appl., 1924, **1**, 125–132 has proposed a blue color reaction with *trioxymethylene* and *gallic acid.* In comparison with calcium strontium and barium show green, magnesium rose and iron brownish violet.

6. With magnesium, but free from all other minerals in muscle, by *electron microscope* (Scott, G. H. and Packer, D. M., Anat. Rec., 1939, **74**, 17–45).

**Camsal** is a mixture of camphor and salol used by McClung in making **Sandarac.**

**Canada Balsam,** see **Balsam.**

**Canary Yellow,** see **Auramin.**

**Cannulas.** Glass cannulas are required for

insertion into blood vessels in the **Perfusion** technique. To make one of about the size for guinea pig's thoracic aorta file and break 6 mm. outside, 4 mm. inside diameter soft glass tubes into pieces about 15 cm. long. (Pyrex of this size will do. It requires a little more heating.) Take one of these, Place middle in gas flame rotating it so as to heat it evenly. When fairly soft remove from the flame, draw the ends apart to a distance of about 50 cm. and hold until solid. File and break in the middle. With a little practice this will give two tubes, each tapering evenly from the 6 mm. outside diameter to about 2-3 mm. within a distance of approximately 3 cm. Next bring the tube where it has a diameter of 2-3 mm. near to a fine flame, like that of a small alcohol lamp. Let it get soft and pull just enough to produce a slight narrowing to be used later to prevent the thread employed to tie the cannula in the vessel from slipping. Then fracture with file and break off the thin end about 4 mm. beyond the constriction and distant from the wide part of the tube. If this break can be made at an acute angle to the length of the tube, so much the better; because then one rim of the small end of the tube will project out beyond the rest which will facilitate its insertion into the vessel to be cannulated. When the break is made across the tube, at right angles, the rim on one side can be ground down on a water stone so as to produce a similarly projecting lip. In either case it is necessary to remove sharp cutting edges from both ends of the cannula by smoothing in a flame. The 6 mm. wide body of the cannula should be 3-4 cm. long for convenient attachment of rubber tube. Obviously larger cannulas are required for larger vessels. Those for **Microinjection** are very much smaller, made of hard glass and do not require to be tied in.

**Capillaries.** In living humans these can best be seen in the skin by the method of Capillaroscopy. Render the epidermis at the root of the finger nail translucent by addition of a drop of highly refractive oil and examine directly at fairly high magnification the capillary loops in the dermal papillae. It is possible to record their changes by making moving pictures through a long period of time. See review by Wright, I. S. and Druryee, A. W., Arch. Int. Med., 1933, **52**, 545-575. See also **Gingiva.**

In living mammals the most favorable site in which to watch capillaries at high magnification is in the transparent chambers of the **Sandison's Technique.** For shorter periods they can be studied in the displaced but living pancreas by the methods of Covell, W. P., Anat. Rec., 1928, **40**, 213-223 and O'Leary, J. L., *ibid*, 1930, **45**, 27-58. Some changes in **Permeability** of living capillaries are evidenced by the trypan blue capillary permeability test. If microdissection is intended and a shift to the tongues and nictitating membranes of frogs is made consult Zweifach, B. W., Anat. Rec., 1934, **59**, 83-108, and Am. J. Anat., 1937, **60**, 473-514. The results have been recorded in moving pictures. Supravital staining of the tissues just mentioned with janus green (Bensley, R. R., and Vimtrup, B., Anat. Rec., 1928, **39**, 37-55) affords beautifully clear views of the muscular elements of arterioles grading into capillaries. See **Perivascular Cells, Rouget Cells.**

For investigations on the topographic arrangement of capillaries arterial injections with **Carmine, Berlin Blue** or some other easily recognizable material followed by clearing by the **Spalteholz** method may be helpful. When however any fluid is injected, under great pressure, into a fresh, relaxed tissue that can easily swell there is a chance that an exaggerated idea of the capillaries will be conveyed. In resting muscle for instance a large proportion of the capillaries are collapsed (Krogh).

The structure of the endothelial capillary wall is relatively uncomplicated. The outlines of the endothelial cells are nicely revealed in pink by the **Silver Chloride Dichlorfluorescineate** technique or in black by simply treating with silver nitrate. Nuclear and cytoplasmic structure can be brought out by methods used for other tissues. Nerve fibers closely accompany most capillaries. The existence of actual nerve endings on the wall is debated. The most convincing looking preparations of human tissues have been secured by Stöhr, Ph., Zeit. f. Zellf. u. Mikr. Anat., 1926, **3**, 431-448 who employed a modification by Gros of the **Bielchowsky** silver technique (see particularly his Fig. 2). See **Sinusoids.**

**Capillaries** of brain. Lepehne-Pickworth peroxidase method simplified by Campbell and Alexander (Mallory, p. 257). Fix for 1-3 weeks in 10% formalin. To make required solution dissolve 0.1 gm. benzidine in 0.5 cc. glacial acetic acid and add 20 cc. aq. dest. Dissolve 0.1 gm. sodium nitroprusside in 10 cc. aq. dest. and add benzidine solution. Add aq. dest. to 100 cc. In case a ppt. forms filter it out. Solution must be fresh. Cut frozen sections 200-300$\mu$ and wash

in aq. dest. 1½ hr. Change to above described solution for ½ hr. at 37°C. agitating often. Wash in aq. dest. 10 sec. and transfer to 100 cc. aq. dest. + 2–3 drops 30% hydrogen peroxide for ½ hr. at 37°C. shaking frequently. Wash in aq. dest. and dehydrate in 70%, 95% and absolute alcohol. Clear in xylol and mount in balsam. Blood vessels black in almost colorless background. This method has the advantage of not involving vascular perfusion.

**Capsule** stain. 1. Hiss' method for smears (McClung, p. 145). Dry organisms in ascitic or serum medium on slides. Stain, slightly heated in 5–10 cc. saturated alc. gentian violet or basic fuchsin made up to 100 cc. aq. dest., few sec. Wash off dye with 20% aq. copper sulphate crystals. Dry by blotting. *See* also: Huntoon, F. M., J. Bact., 1917, **2**, 241.

2. W. H. Smith's method for sections (Mallory, p. 275). Cover deparaffinized sections of Zenker fixed material with **Anilin Crystal Violet** (either Ehrlich's or Stirling's). During few seconds warm by passing slide through flame 2 or 3 times. Wash in **Gram's Iodine** solution followed by formalin (commercial). Decolorize in 95% alc. Quickly wash again in Gram's iodine. Cover with aniline green eosin and heat as before. To make this shake 1 part aniline green with 200 parts 3–6% aq. eosin yellowish W.S. and after 1–2 hrs. remove ppt. by filtering. Wash in aq. dest. Dehydrate in 95% and abs. alc., clear in xylol and mount in balsam. Bacterial capsules, red; Gram positive bacteria, blue. Mallory says that a stronger iodine may be desirable (iodine, 1 gm., potassium iodide, 2 gm.; aq. dest., 100 cc.) and that the times must be suited to each preparation.

**Carbol-Anilin Fuchsin** methylene blue method for Negri bodies (Goodpasture, E. W., Am. J. Path., 1925, **1**, 547–582). Fix in Zenker's fluid 24 hrs. (not Helly's fluid). Color for 10–30 min. in mixture made by adding 1 cc. of pure phenol and 1 cc. of anilin oil to 100 cc. of stock 0.5% basic fuchsin in 20% alcohol. Wash in running water, blot with filter paper and decolorize with 95% alcohol until sections become pink. Then wash in water and stain with **Loeffler's** methylene blue, 15–60 sec. Wash again in water. Dehydrate and destain for few sec. in absolute alcohol, clear in xylol and mount in balsam. Negri bodies, crimson; background, blue. Also excellent for **Borrel Bodies.**

**Carbol-Fuchsin.** The original formula of Ziehl has been much modified. Ziehl-Neelsen is sat. alc. basic fuchsin, 10 cc.; 5% aq. carbolic acid, 90 cc. Verhoeff (F. H., J.A.M.A., 1912, **58**, 1355) advises basic fuchsin, 2 gm.; abs. alc., 50 cc.; melted carbolic acid crystals, 25 cc. McClung (p. 136) suggests mixing 10 cc. 3% basic fuchsin (90% dye content) with 95 cc. 5% aq. phenol. The important thing is the character of the fuchsin not its concentration relative to carbolic acid. Carbol-fuchsin is employed in stains for **Acid Fast Bacilli.** Deipolli, G. and Pomerri, G., Mon. Zool. Ital., 1938, **49**, 123–124 have advised its use as follows for **Nissl Bodies.** Fix small pieces in 95–98% alcohol or in 10% formalin water or in physiological saline 24 hrs. or longer. Stain deparaffinized sections 3–4 min. in carbol-fuchsin (basic fuchsin, 0.2 gm.; conc. phenol, 1 cc.; 95% alc., 2 cc.; aq. dest. 20 cc.) 2.5 cc.; aq. dest., 100 cc.; glacial acetic acid, 0.5 cc. Wash rapidly in aq. dest. and destain in: aq. dest., 100 cc.; formalin, 1 cc.; glacial acetic acid, 1 cc. Wash in aq. dest., dehydrate in alcohols, clear in xylol and mount in neutral balsam. Nissl bodies and nucleoli dark red, rest unstained.

**Carbol-Thionin,** see **King's.**

**Carbol-Xylol.** Xylol saturated with carbolic acid crystals. After using it for clearing celloidin sections, wash quickly in xylol before mounting them in balsam.

**Carbon** from inspired air occurs abundantly in lungs and bronchial lymph nodes. It may be transported to the great blood filters (spleen and liver) where it is distinguishable by its black color and by its insolubility in conc. sulphuric acid which dissolves all other body pigments. Fine suspensions of carbon are of great service as vital stains to demonstrate phagocytosis. See **Higgins' Ink** and **Lampblack.**

**Carbonic Anhydrase.** This can be localized in the oxyntic (or parietal) cells of the fundus of the stomach. Davenport, H. W., Am. J. Physiol., 1940, **128**, 725–728; **129**, 505–514 employed an adaptation of Linderstrøm-Lang's technique and observed that in rats and cats the parietal cells contain 5 to 6 times as much of the enzyme as red blood cells while the peptic cells are free from it. A microspectroscopic method for demonstration of carbonic anhydrase within erythrocytes depends on the action of methemoglobin as an indicator which changes both its color and pattern of absorption spectrum with change of pH from 6.5–9.5 (Keilin, D. and Mann, T., Nature, 1941, **148**, 493–496). For data on the distribution of this enzyme in lower forms, see Blaschko and Jacobson (Bourne, p. 200).

**Carey's** method for motor end plates is an adaptation for his study of their ameboid motion (Carey, E. J., Anat. Rec., 1941, 81, 393–413) of Wilkinson's (H. J., Med. J. Austral., 1929, 2, 768–793) modification of *Ranvier's gold chloride technique.* Cut out pieces of rat's intercostal muscle 3–5 mm. thick with sharp surgical scissors. Fix immediately in full strength, fresh, filtered lemon juice for 10–15 min. Decant lemon juice and, without any washing in aq. dest., pour on 1% aq. gold chloride made up 1 or 2 days before use. Keep in dark until tissue takes uniform golden color 10 min. to 1 hr. Do not leave until brown. Again, without washing in aq. dest. transfer tissue to 1 part formic acid + 3 parts aq. dest. in dark for 12 hrs. (If the tissue was cut sufficiently thinly and uniformly just enough solution penetrates and is reduced to color the muscle reddish blue and the nerve components black.) Wash once in tap water and quickly place in equal parts 50% alcohol and chemically pure glycerin. Remove a piece of tissue, place in drop of glycerin on a slide, orient with teasing needles, gently spread with cover glass and ring edges with *Clarite X.* See Carey's beautiful photographs.

**Carmalum** (Mayer). Dissolve, if necessary with heat, 1 gm. Carminic acid and 10 gms. ammonia alum in 200 cc. aq. dest. Filter and to filter add 1 cc. formalin as a preservative. The tissues stained should not be alkaline (Lee, p. 141).

**Carmine** has been very widely used as a stain. Most of the formulae for staining of fixed tissues were proposed 40 or more years ago chiefly by Ranvier and Mayer. Now aniline dyes are more popular but carmine is still of great use for staining small animals *in toto*, for staining tissues in bulk which are later sectioned, as the best counterstain for blue vital dyes like trypan blue, as the most specific stain for **Glycogen** and for **Mucus** in the form of mucicarmine, for coloring gelatin used to inject blood vessels and as a vital stain. Karsner, H. T. and Swanbeck, C. E., J. Med. Res., 1920, 42, 91–98 employed 15–25 cc. of fairly thick suspension for intrapleural injections in cats. At present carminic acid is available and can be employed instead of powdered carmine. The only advantage is that the acid is of more uniform composition. See **Acetocarmine** (Schneider), **Alum Carmine** (Grenacher), **Aluminum Chloride-Carmine** (Mayer), **Ammonia Carmine** (Ranvier), **Best's Carmine** for glycogen, **Borax Carmine** (Grenacher), **Carmalum** (Mayer), **Lithium Carmine** (Orth), **Mucicarmine** for mucus, **Para-Carmine** (Mayer), **Picro-Carmine** (Ranvier). Many more carmine combinations are given by Lee (pp. 139–149).

**Carmine-Gelatin Injections** of blood vessels. Methods have been reviewed by Moore, R. A., J. Tech. Methods, 1929, 12, 55–58. He proposes a more accurate technique for preparation of the gelatin mass. Allow 80 gms. gelatin to take up 200 cc. cold water and heat to complete the gel. Suspend 20 gms. carmine in 100 cc. water and add ammonia until dissolved. Mix the gelatin and carmine solutions and add 15 gms. potassium iodide to reduce gelation point to less than 25°C. Place in water bath at 25°C. and immerse a prepared platinum electrode in it. Pass electrolytic hydrogen from a tank over the electrode and agitate the gelatin with a motor stirrer. Read electrical potential by balancing against a standard cell. Add acetic acid cautiously until reading of voltage corresponds to pH 7.2.

Two other techniques are listed by Moore: 1. Dissolve 40 gms. carmine in 40 cc. strong ammonia and add water. Allow to stand 12–24 hrs. and filter through paper. Boil filtrate until it is ammonia free. Precipitate the carmine as a colloidal gel by adding 95% alcohol. Filter, wash well with alcohol and dry material collected. Dissolve 2 gm. in 5 cc. water and add 5 cc. 100 percent gelatin in water thus making the product 20% carmine and 50% gelatin (Bensley, R. R., personal communication to Dr. R. A. Knouff). 2. Triturate 40 gms. carmine Merck NFIV with 40 cc. strong ammonia and add water to 200 cc. After standing 24 hrs. filter through paper. Boil filtrate down to 100 cc., add water to 200 cc. and repeat. Add 70 gms. gelatin dissolved in water and make up with water to 1 liter (MacCallum, D. B., Am. J. Anat., 1926, 38, 153–175).

**Carnoy-Lebrun** fixative for insects and ticks. Equal parts chloroform, absolute alcohol and acetic acid saturated with mercuric chloride. See **Slifer-King Method.**

**Carnoy's Fluid** in abs. alc., 6 parts; chloroform, 3 parts; and glacial acetic acid, 1 part. Also known as Van Gehuchten's mixture. A very quick fixative. Do not wash in water but in 95% alc. It is employed for many purposes. See **Fibrin, Foot's Method, Glycogen Neurofibrils.**

**Carotin,** put green leaves in sat. aq. KOH, 1 part; 40% ethyl alcohol, 2 parts and tap water 3 parts in wide mouthed bottle with tight glass stopper to prevent absorption of $CO_2$ from air or seal with vaseline. Keep several days in dark until tissue is yellow and fluid is green. Change pieces to aq. dest. several hours.

Remove small pieces, dry on slide with filter paper. Add 1 drop conc. $H_2SO_4$. It turns green, then blue. Under microscope carotin crystals appear dark blue (Steiger, A., Microkosmos, 1941, **8**, 121–122). Carotin is a precursor of **Vitamin A.**

**Carotinalbumins.** Combinations of carotinoid pigments with protein. Rather uncommon. As an example Lison (p. 245) cites the blue carotinalbumin in the carapace of the lobster which on boiling is split into a protein and a red carotinoid.

**Carotinoids.** Pigments which are nonsaturated and nonnitrogenous hydrocarbons. Entirely different chemically from fats, they are nevertheless only present *in vivo* as solutions within lipoids. They generally appear yellow, orange or brown in unstained frozen sections mounted in syrup of levulose. Lison (p. 244) indicates that tissues containing these pigments can sometimes be imbedded in paraffin, because they are only slowly soluble in cold alcohol. They are however more quickly soluble in chloroform, acetone petroleum ether and toluol. According to Lison (p. 245) they are always easily identifiable by the fact that when treated with concentrated sulphuric acid they turn intense blue before being destroyed. Treated with solution of iodine-iodide (say Gram's, Lugol's) they give a black green or brown color. When treated with solution of chromic acid they lose their color more or less quickly. See **Lipids,** tabular analysis, also **Carotin.**

**Carr-Price Reaction** for vitamin A. When frozen sections of liver are plunged directly into a solution of antimony trichloride in chloroform and immediately examined therein mitochondria take bright blue color which fades within 30 min. (Bourne, G., Austral. J. Exp. Biol. & Med. Sci., 1935, **13**, 238–249). Antimony trichloride is said not to be specific for vitamin A since it also gives blue color with carotinoid pigments (Bourne, p. 106).

**Cartilage.** This is one of the most awkward tissues of the body to examine in the living state because of the mechanical difficulties involved in separating its component parts sufficiently thinly for examination at high magnification in approximately isotonic media. But the differentiation of cartilage in tissue cultures has been studied to advantage (Fell, H. B., Arch. f. exper. Zellf., 1929, **7**, 390–412) and an account of the direct investigation of living cartilage in **Sandison** transparent chambers inserted in the ears of rabbits (Clark, E. R., and E. L., Am. J. Anat., 1942, **70**, 167–200) sounds very promising. The varieties of cartilage (hyaline, articular, elastic and fibrous) depend upon the quantitative and qualitative differences in the three chief components—cells, fibers and ground substance.

When the cartilage is fixed to bone, which is also to appear in the sections, it is obviously necessary to employ decalcification, see **Bone.** Otherwise cut thin slices, 2–4 mm. thick, and fix by immersion. Fixation by perfusion is not a great help because cartilage is practically avascular. The choice of fixatives and stains will depend upon what it is desired to demonstrate. For routine purposes **Zenker's Fluid** is satisfactory followed by coloration of paraffin sections with **Hematoxylin** and **Eosin** or **Mallory's Connective Tissue** stain. But many prefer Celloidin sections. **Resorcin Fuchsin** is recommended for the elastic fibers of the matrix. Since the *fibers* are somewhat obscured by the ground substance in hyaline cartilage dark field and polarized light may be useful as employed by Lubosch, W., Zeit. f. mikr. Anat., Forsch., 1927, **11**, 67–171. A paper by Dawson, A. B., and Spark, C., Am. J. Anat., 1928, **42**, 109–137 also contains useful information. If it is desired to show the Golgi apparatus in the *cells* follow the technique used by Fell, H. B., J. Morph., 1925, **40**, 417–459. See **Chondriotin Sulphuric Acid** and **Phosphatase** as components of cartilage. The specific staining of cartilage cells with crystal violet has been reported by Hass, G. M., Arch. Path., 1942, **33**, 174–181. The characteristic basophilia of the ground substance is the basis for the following excellent method for the demonstration of cartilage in whole mounts.

*Van Wijhe's methylene blue* (Noback, G. J., Anat. Rec., 1916–17, **11**, 292–294). This, by demonstrating cartilage in blue in transparent whole mounts, supplements very nicely the vital coloration of growing bone by **Madder** feeding or **Alizarin** injections. Use embryos, or bones of young animals like rats or mice, long bones, ribs, chrondocranium, etc. Fix in 10% formalin a day or more. 1% hydrochloric acid in 67% alcohol several days or a week. Same solution + 0.25% methylene blue or toluidin blue 1 or 2 weeks until thoroughly stained. Decolorize in **Acid Alcohol.** Change alcohol when it becomes much colored or every 1 or 2 days. Continue until only the cartilage retains deep blue color. Wash several days in 82% alc. Dehydrate in 95% and abs. Equal parts abs. and benzene. Benzene change twice.

Leave in this or mount in xylene damar which is better than balsam because of its light color.

**Cartilaginous Skeleton** of mammalian fetuses. A modification of the Wijhe, Lundvall and Schultze techniques used in the Department of Embryology, Carnegie Institution of Washington is given by Miller, C. H., Anat. Rec., 1921, **20,** 415–419. Wash formalin fixed material over night in water plus few drops ammonia. Transfer to 70% alcohol and leave 7–14 days changing alcohol daily for first five. Stain for 3–10 days in: toluidin blue (Grubler), 1 gm.; 70% alcohol, 400 cc.; and hydrochloric acid, 4 cc. Decolorize for 7–10 days until decolorizer is but slightly tinged with the dye in: 70% alcohol, 100 cc. plus hydrochloric acid, 1 cc. Then 80% and 95% alcohol, 3 days each. Transfer to 2% potassium hydroxide, in aq. dest. and leave 2–3 days until cleared. Change to 20, 40, 60, and 80% glycerin in aq. dest. 2 days or more in each. Store or mount in pure glycerin plus few crystals of thymol. Obviously length of times depends chiefly upon size of specimen. This staining of cartilage with toluidin blue can be combined with the coloration of bone with **Alizarin Red S** to make very contrasty preparations (Williams, T. W., Stain Techn., 1941, **16,** 23–25).

**Caseation** (L. *caseus*, cheese). This change follows local **Necrosis.** It is characterized by grayish or light yellow cheesy masses of tissue which look amorphous and have lost their original structure. Identification is morphological. Almost any good staining method is satisfactory. In some cases fibrin is present.

**Catalase.** Method for demonstration in elementary bodies of vaccine virus (Macfarlane, M. G., and Salaman, M. H., Brit. J. Exp. Path., 1938, **19,** 184; Hoagland, C. L. et al., J. Exp. Med., 1942, **76,** 163–173).

**Cataract,** see **Optic Lens.**

**Cathepsin.** A method for analysis of cathepsin in lymphocytes and polymorphonuclear leucocytes (neutrophiles) is given by Barnes, J. M., Brit. J. Exp. Path., 1940, **21,** 264–275.

**Cedar Oil,** see **Clearing** and **Mounting.**

**Celestin Blue B** (CI, 900)—coreine 2R—A basic quinone-imine dye employed by Proescher, F. and Arkush, A. S., Stain Techn., 1928, **3,** 28–38 and by Lendrum, H. C., J. Path. & Bact., 1935, **40,** 415–416 as a nuclear stain.

**Cell-Division,** see **Mitosis, Amitosis** and series of papers on chemistry of cell division (Mauer, M. E. and Voegtlin, C., Am. J. Cancer, 1937, **29,** 483–502).

**Cell Injury** detected by fluorescence (Herick, F., Protoplasma, 1939, **32,** 527–535). See **Dead Cells.**

**Cell Membranes** do not require any special technique for their demonstration. Almost any good fixative will do and they can be stained a host of different colors. There is however some difference in the interpretation of what we see with the microscope. The essential component of the walls of all cells is called the *plasma membrane*. This conditions permeability and its integrity is essential to the life of the cell. It is said to consist of a continuous layer of lipoid molecules (phosphatides, sterols, fats) not more than 2–4 molecules thick on which proteins are adsorbed, the lipoids give permeability and the proteins elasticity and great mechanical strength. The evidence is critically presented by Danielli (Bourne, pp. 68–98). He says that it is improbable that the lipoid layer is ever thicker than 10 m$\mu$ and that the whole membrane is between 1$\mu$ and 1 m$\mu$ thick. Consequently in many cases we cannot expect to visualize the plasma membrane itself directly with visible light because the theoretical limit of visibility is a particle size of 0.25$\mu$. However the position of the plasma membrane is made clear by the difference in properties of the cytoplasm which it limits and the fluid without and also in the dark field by the light reflected from its surface. In addition it is often backed internally by a thin layer of cytoplasmic cortex (ectoplasm) which is typically free from cytoplasmic granules. The plasma membrane may be supplemented externally by special membranes such as the myelin sheaths about nerve fibers. There are many special techniques for its investigation. Some are briefly referred to under **Lysis, Permeability, Surface Tension** and **Wetting Properties, Nuclear Membrane, Pinocytosis.**

**Celloidin Imbedding.** Celloidin is a kind of generic term covering various cellulose compounds, nitrocellulose, soluble gun cotton, etc., employed for imbedding. The collodions are solutions of pyroxylin made as specified in the U.S.P. Pyroxylin U.S.P. XI consists chiefly of cellulose tetranitrite (Merck Index, p. 465). Obviously a purified, nonexplosive form of pyroxylin is necessary. There are several in the market of which Parlodion (Mallinckrodt) is the one used in our laboratory. The Bensleys (p. 37) use as celloidin "RS ½ sec. low viscosity nitrocellulose 30 per cent solvent in absolute alcohol" obtained from the Hercules Powder Co., Gillespie, N. J. To make 20% stock solution they dissolve 140 gms. nitro-

cellulose in 250 cc. ether and 210 cc. absolute alcohol. This requires 4–5 days shaking occasionally. It is diluted with ether alcohol to make 10 and 5% solutions respectively. Nitrocellulose is much used especially in neurological technique. It is abbreviated **L.V.N.** Some advantages over "celloidin" are claimed for it by Davenport, H. A., and Swank, R. L., Stain Techn., 1934, **9**, 137–139.

Celloidin imbedding is less popular than it used to be owing to certain advantages of **Paraffin Imbedding** reinforced by the mania for speed. But celloidin imbedding is in some respects superior. It yields sections in which the affinity of the tissue components for dyes is often greater. Clearing of the tissue in xylol and similar fluids is not required and it need not be subjected to heat. The tissue usually shrinks less and seldom becomes so brittle. Brain specimens can easily be cut in celloidin even after long mordanting. When sections are required of large pieces of tissue in which cavities, such as the lumina of the paranasal sinuses, alternate with stout bony walls this method is indicated because the celloidin in the spaces gives more support than paraffin (see also **Double Imbedding**).

The slow method, which is the best, requires for tissue slices not more than 5 mm. thick, at least 1 day each in 95% alcohol, absolute alcohol, and in half absolute and ether. This is followed by 1 day in thin celloidin (about 4% dried strips of celloidin—Parlodion, Mallinckrodt—dissolved in equal parts absolute alcohol and ether) and 1 or more weeks in thick 8% celloidin. The tissue, with some celloidin about it, is then mounted on a fiber block, hardened in chloroform 1–2 hrs. and stored in 80% alcohol.

Mallory (p. 60) gives the following as a rapid method. Fix thin tissue pieces 12–18 hrs. in **Formalin-Alcohol.** Then 95% alcohol, 2 changes, 2 hrs.; absolute alcohol, 2 changes, 3 hrs.; alcohol-ether, 3 hrs.; thick celloidin 12–15 hrs.; mount and harden in chloroform, 1 hr.; 80% alcohol.

A *still quicker* technique has been proposed (Richardson, G. D., J. Tech. Meth., 1934, **13**, 81): To make celloidin solution, add 1100 cc. absolute ethyl alcohol to 8 oz. celloidin (dried in air) and leave over night. Add 1100 cc. ether. Let stand several days. It is ready when celloidin is dissolved. Fix tissue in 10% formalin, 2 hrs.; acetone, 2 hrs.; oil of cloves ½–2 hrs. or until clear; celloidin 6 hrs. at room temperature or ½–3 hrs. in water bath at 55°C. (being careful to keep away from flame). Block and harden in chloroform ½–2 hrs.

Another so called *hot celloidin* method is proposed with all steps in the technique at an elevated temperature (Koneff, A. A., and Lyons, W. R., Stain Techn., 1937, **12**, 57–59). Fix pieces not thicker than 2–3 mm. in 10% neutral formol, **Bouin** or **Susa.** Wash in aq. dest. several changes (1 hr. each) at room temperature. Dehydrate at 50°C. 70, 80, 95 and abs. alc. 2 changes ½ hr. each. Equal parts abs. alc. and ether 1 hr. Infiltrate at 56°C. in (1) 10% nitrocellulose (R.S. ½ second, viscosity $\frac{3}{4}$–$\frac{1}{2}$, Hercules Powder Co.) in equal parts abs. alc. and ether, 1 hr. (2) 25% in 45 cc. alc. + 55 cc. ether, over night. (3) 50% in 40 cc. alc. + 60 cc. ether 2–3 hrs. Then transfer tissue to microtome block moistened with ether-alcohol. Add 50% nitro-cellulose and the tissue. Harden in 2 changes chloroform during 1 hr. Then pass through 3 changes 80% ethyl alcohol and cut. The authors mention fixation in "Carnoy II" and removal of mercury with iodized alcohol in case a fixative containing mercuric chloride was employed. Obviously every precaution must be taken to avoid explosion.

Store celloidin blocks in 80% alc. See special methods for imbedding **Teeth** and **Bone.**

**Celloidin Injections** of lungs. For smaller vessels and bronchi use: acetone, 100 cc.; celloidin, 4 gm.; and camphor, 3 gm. For larger vessels and bronchi employ: acetone, 100 cc., sheet celloidin, 20 gms., and camphor, 15 gm. In place of sheet celloidin old x-ray films can be used if first the emulsion is removed by washing in warm water and they are then dried and cut into strips. If colors are desired employ oil paints. If Roentgenograms are to be made of the corrosion specimens add 10–12% sodium iodide or barium sulphate to a 30% suspension. In case of the vessels wash out blood first by forcing physiological saline solution into vena cava thence through right heart and via pulmonary arteries to lungs evacuating by pulmonary veins. Allow injected lung to stand in running water over night thus hardening celloidin. Immerse in concentrated hydrochloric acid to digest away tissues leaving celloidin cast. This usually takes 24 hrs. Wash thoroughly in gentle stream of water. Mount dry or mount wet in solution made up as follows: Boil for 10 min. 100 cc. aq. dest. + 20 cc. glycerin. When cool add formalin to 2% and filter until clear (Marquis, W. J., J. Tech.

Methods, 1929, **12**, 59-64). See illustrations of Marquis and arrangement of pressure bottles. A celluloid corrosion technique for the kidney is described by N. W. Baker, J. Tech. Methods, 1929, **12**, 65-68.

**Celloidin Sections.** Cut side of celloidin block to smooth plane surface. Moisten this and surface of microtome block holder with alcohol-ether. Add drop thick celloidin. Press together, harden in chloroform and cut in 80% alcohol on a sliding microtome with knife at an angle. Keep surface of knife and block wet with 80% alcohol from overhead dropping bottle. (A method has been described for treating block with cedar oil and cutting dry with rotatory microtome, Walls, G. L., Stain Techn., 1936, **11**, 89-92). Sections are usually cut at a thickness of 10-16 $\mu$. (It is possible to arrange the sections serially but it is a tedious business. If serial sections are needed, paraffin should be selected in place of celloidin.) The sections unmounted can be stained without removing the celloidin after which they are to be dehydrated and cleared before mounting. The object is not to remove the celloidin but to soften it. The following mixture is recommended by Lee (p. 108) in place of xylol, toluol or benzol: creosote, 40 cc.; Bergamot oil, 30 cc.; xylol, 20 cc. and origanum oil, 10 cc.

**Cellosolve** is ethylene glycol monoethyl ether. It mixes with water, acetone, alcohol, ether and dissolves many oils, waxes, etc. Employed by Lendrum (A.C., J. Path. & Bact., 1939, **49**, 590-591).

**Cellulose,** microchemical reaction for. Solution A: Dilute 20 cc. of 2% iodine in 5% aq. potassium iodide with 180 cc. aq. dest., add 0.5 cc. glycerin and mix by shaking. Solution B: Saturate 15 cc. aq. dest. with lithium chloride at 80°C., cool and use supernatant solution. Tease out section or fibers. Apply 2-3 drops "A" by glass rod and leave 10 sec. Blot with filter paper and dry. Add drop "B", cover and examine. Cellulose blue, green, yellow depending upon its source (Post, E. E. and Laudermilk, J. D., Stain Techn., 1942, **17**, 21-26.

**Cement** for ringing specimens mounted in glycerin, etc. See **Kronig's** cement and **Mounting Media.**

**Centigrade** temperature to Fahrenheit

1. Above 0°C. multiply by 9, divide by 5, add 32. *Example:* 37°C. = 37 × 9 = 333 ÷ 5 = 66.6 + 32 = 98.6°F.
2. Between −17.77 and 0°C. multiply by 9, divide by 5 subtract from 32. *Example:* −12°C. = 12 × 9 = 108 ÷ 5 = 21.6; 32 − 21.6 = 10.4°F.
3. Below −17.77°C. Multiply by 9, divide by 5, subtract 32. *Example:* −18°C. = 18 × 9 = 162 ÷ 5 = 32.4 − 32 = 0.4°C.

**Central Body,** see **Centrosome.**

**Centrifugation.** To even sketch in outline the techniques that come under this heading is difficult because the centrifugation of so many materials and tissues is helpful and the instruments vary from simple hand driven machines to powerful ultracentrifuges which may weigh several tons and which certainly require experts to care for them. See Svedberg, T. and Pedersen, K. O., The Ultracentrifuge, Oxford, Clarendon Press, 1940, 478 pp.

The centrifuge has long been of help in the displacement of certain components of cells (especially marine eggs) in order to determine their functional rôles. It has also proved invaluable in the investigation of cytoplasmic and nuclear **Viscosity,** which see.

In recent years centrifugation has opened a new chapter in microchemistry by the part which it has played in the collection of cellular components in sufficient volume for analysis. Pioneer work was done with the liver. The Bensleys (p. 6) give instructions which are in part as follows. First perfuse the abdominal organs of a guinea pig with about 1000 cc. 0.85% aq. sodium chloride (see **Perfusion**). This removes a good deal of the blood. Excise liver and grind up thoroughly in a mortar. Place the resulting thick fluid in large centrifuge tubes, add about twice the volume of 0.85% aq. sodium chloride and balance the tubes with more as may be necessary. If complete separation of mitochondria is desired centrifuge for 1 min. at 3000 r.p.m. which results in stratification. In first and lowest stratum, at the bottom of the tubes, will be found liver cells, cell debris and connective tissue elements; in the second, nuclei and red blood cells; in the third mitochondira and small cell fragments; and in the fourth and uppermost, free fatty droplets. The materials in any of these layers can then be collected by drawing up in a pipette, suspended again in salt solution and purified by further centrifugation.

For the isolation of *ellipsin* (structural protein) and *mitochondria* see Bensley, R. R. and Hoerr, N. L., Anat. Rec., 1934, **60**, 251-266 and 449-455. Since it is in the mitochondrial fraction resulting from centrifugation that *vitamin A* is found the Goerners have greatly extended the usefulness of the method in a series of studies on tumors (Goerner)

A., J. Biol. Chem., 1937-38, **122**, 529–538 and A., and M. M., *ibid*, 1939, **128**, 559–565). The technique has been further improved by Claude (A., Science, 1938, 87, 467–468; Cold Spring Harbor Symposia on Quantitative Biology, 1941, **9**, 263–270) who used 18000 r.p.m. See, particularly, standardized techniques in his 1941 paper. Beams, H. W. and King, R. L., Anat. Rec., 1940, **76**, 95–101, and in a series of other papers, have greatly contributed to the use of ultracentrifugation in the solution of biological problems. See Lucas, A. M., Am. J. Path., 1940, **16**, 739–760 on intranuclear inclusions.

**Centrifuge Microscope** is an instrument whereby the behavior of living cells can be investigated at high magnification while they are being centrifuged. It is of great service in the determination of what actually happens at different times and at different speeds. The displacement of granules in living cells can be actually watched. Details are supplied by E. B. Harvey in McClung (pp. [illegible]

**Centriole,** see **Centrosome.**

**Centrosomes** (G. *Kentron*, center; *soma*, body), sometimes called a "central body", is a minute spherule which is a dynamic center of some sort involved in cell division. It is sometimes called a *centriole* though Conklin (Cowdry's General Cytology, pp. 542 and 544) says that a central body, the *centriole*, appears within the centrosome during mitosis. When the centrosome is double, that is consists of two minute bodies side by side, it is designated a *diplosome*. About the centrosome, or diplosome, there is usually a clear area which is known as a *centrosphere*. The centrosome, or centriole plus the clear area is called the *cytocentrum*. For terminology see Wilson, E. B., The Cell. New York: Macmillan Co., 1925, 1232 pp. For functional significance see Fry, H. J., Biol. Bull., 1929, **57**, 131–150. Giant centrospheres in degenerating cells are described by Lewis and Lewis (Cowdry's General Cytology, p. 427) and multiplication of centrioles in striated muscle tumors by Wolbach, E. B., Anat. Rec., 1928, **37**, 255–273.

Centrosomes are not easily demonstrated in tissue sections. The technique originally used by Heidenhain (Arch. f. mikr. Anat., 1894, **42**, 665) appears to be the best. It consists of fixation in a **Sublimate Acetic,** or **Sublimate Alcohol Acetic,** and of staining the sections 24 hours in a dilute aq. sol. of Bordeaux red or of anilin blue followed by iron hematoxylin in the usual way. The centrosomes are stained black or gray with a tinge of red or blue. In glandular epithelial cells look for them in the cytoplasm between the nucleus and the lumen.

To reveal centrosomes in non-dividing nerve cells is difficult, probably because they are seldom present. Hatai (S., J. Comp. Neurol., 1901, **11**, 25) was able to stain them in certain nerve cells of adult rats. He fixed in sat. mercuric chloride in formalin, 30 cc.; glacial acetic acid, 50 cc. and physiological salt solution, 15 cc. for 6–12 hrs., then washed, 4–5 hrs. in running water, imbedded in paraffin, stained in sat. aq. toluidin blue or thionin, dehydrated, cleared and mounted the sections. Rio Hortega (P., Trab. Lab. Invest. Biol. Univ. Madrid, 1916, **14**, 117) has obtained beautiful silver preparations of centrosomes. Addison (McClung, p. 469) advises fixation in **Flemming's Fluid** or in Allen's chromic-urea modification of **Bouin's Fluid** followed by staining with Heidenhain's **Iron Hematoxylin.**

A detailed investigation of the effects of a great many fixatives on the mitotic figure in chaetopterus eggs has been made by Fry (Fry, H. J. Biol. Bull., 1933, **65**, 207–237). He concluded (1) that acetic acid, picric acid, formaldehyde and alcohol and certain combinations of them are most useful as fixatives (2) that anesthetics like chloroform and ether and inorganic fixatives are to be avoided; (3) that the fixatives must be diluted to about 10% of the original concentration with aq. dest. or better with sea water. Comparable information for human tissues is lacking.

**Cephalin,** a phosphatide, is a compound of phosphoric acid, glycerol, 2 fatty acid molecules and amino ethyl alcohol. It differs also from lecithin in being only very slightly soluble in alcohol, see **Lipoids.**

**Cerasin R,** see **Bordeaux Red.**

**Cerasin Red,** see **Sudan III.**

**Cerebrosides** are galactosides, that is compounds of fatty acid, galactose and sphingosine, without phosphorus, soluble in benzene, pyridine and hot alcohol and almost insoluble in ether, see **Lipoids.**

**Ceresin Imbedding.** Ceresin is purified ozokerite, a mixture of hydrocarbons, with melting point 61–78°C. used as a substitute for beeswax and for other purposes. Waddington, C. H. and Kriebel, J., Nature, 1935, **136**, 685 advise for hard objects like feathers addition of ceresin to a paraffin of slightly lower melting point than that usually employed. The whole, when cooled, has

a very fine texture. See the methyl benzoate celloidin ceresin method of 'Espinasse for imbedding hard objects in a suitable condition for sectioning as described by Lee (p. 96) and Waterman, H. C., Stain Techn., 1939, **14**, 55–62.

**Cerotine Ponceau 3B,** see **Sudan IV.**

**Cesares-Gil** flagella stain evaluated, Thatcher, L. M., Stain Techn., 1926, **1**, 143–144.

**Cesium,** spectrographic analysis of, in retina (Scott, G. H. and Canaga, B., Jr., Proc. Soc. Exp. Biol. & Med., 1939, **40**, 275).

**Cestoda,** see **Parasites, Taenia.**

**Cevitamic Acid,** see **Vitamin C.**

**Champy-Kull's Method** of anilin fuchsin, toluidine blue and aurantia for mitochondria. Fix in Champy's fluid (3% potassium bichromate, 7 cc.; 1% chromic acid, 7 cc.; 2% osmic acid, 4 cc.) 24 hrs. Wash in aq. dest. Place in pyroligneous acid, 1 part and 1% chromic acid, 2 parts 20 hrs. Wash aq. dest. 30 min.; mordant 3% aq. potassium bichromate, 3 days. Wash running water 24 hrs., dehydrate, clear, imbed and section at 4$\mu$. Remove paraffin from sections. Stain with anilin acid fuchsin (acid fuchsin 10 gms., anilin water 100 cc.) heated over spirit lamp and allow to cool 6 min. Rinse in aq. dest. Counterstain in 0.5% aq. toluidine blue 1–2 min. Rinse in aq. dest., then 0.5% aurantia in 70% alcohol 20–40 sec. Differentiate in 95% alcohol, dehydrate, clear and mount. Mitochondria red, nuclei blue and ground substance yellow.

**Champy's Fluid** is 3% potassium bichromate, 7 parts; 1% chromic acid, 7 parts; and 2% osmic acid, 4 parts. It is an excellent fixative for cytologic details.

**Cheese.** Bacteria in, see Hucker, G. J., N. Y. Agric. Exp. Sta. Tech. Bull. 1921, 87 (McClung, p. 147).

**Chicago Blue,** see escape from venules after intravenous injection (Smith, F. and Rous, P., J. Exp. Med., 1931, **54**, 499–514).

**China Blue,** see **Anilin Blue.**

**Chitin.** 1. Method for softening of chitin in formalin fixed insects (Murray, J. A., J. Roy. Micr. Soc., 1937, **57**, 15). Fix primarily in 10% formalin in 0.8% aq. sodium chloride, or indefinitely. Fix secondarily and dehydrate in equal parts absolute alcohol, chloroform and glacial acetic acid + corrosive sublimate to saturation (about 4%). Warm together equal parts chloral hydrate and phenol until they fuse and form an oily liquid which is fluid at room temperature. Leave specimens in this 12–24 hrs. or longer. Clear in chloroform, xylol or carbon disulphide. Imbed in paraffin.

2. According to Hennings (see Lee, p. 597) fixation of insects in the following mixture softens the chitin sufficiently to permit the making of paraffin sections: nitric acid, 16 cc.; 5% aq. chromic acid, 16 cc.; sat. corrosive sublimate in 60% alcohol, 24 cc.; sat. aq. picric acid, 12 cc.; and abs. alc., 42 cc. Fixation is 12–24 hrs. followed by washing in iodine alcohol. An older method is to soften chitin by treatment with a solution of hypochlorite of soda (Lee, p. 249). See **Diaphanol, N. Butyl Alcohol, Insects,** and **Ticks.**

**Chloral Hydrate,** as a fixative for peripheral nerves (Bank, E. W. and Davenport, H. A. Stain Techn., 1940, **15**, 9–14). Chloral hydrate is also recommended as a macerating medium for the separation and isolation of epithelial and lining cells by the Bensleys (p. 5). According to their instructions remove small pieces alimentary tract of pithed or freshly killed frog and leave them in 5% aq. chloral hydrate 12–48 hrs. Then tease with fine needles and examine. See **Cajal's** chloral hydrate method.

**Chlorazol Black E** (CI, 581) of British Dyestuffs Corporation—Erie black G X 00 (National Aniline and Chemical Company), Pontamine black E (I. E. Du Pont deNemours & Co.)—an acid poly-azo dye. First described as a new biological stain by Cannan (H. G., Nature, 1937, **139**, 549). Review of its uses (Cannan, H. G., J. Roy. Micr. Soc., 1941, **61**, 88–94). As a vital dye (Baker, J. R., Nature, 1941, **147**, 744). Stains chromatin black, cytoplasm greenish gray after Zenker fixation (Darrow, M. A., Stain Techn., 1940, **15**, 67–68). As an aceto-carmine auxillary stain for chromosomes (Nebel, B. R., Stain Techn., 1940, **15**, 69–72).

**Chlorazol Blue 3B,** see **Trypan Blue.**

**Chlorazol Pink Y,** see **Thiazine Red R.**

**Chloride.** In 1908 Macallum reviewed the older literature and described his silver test for chloride (Macallum, A. B., Ergeb. d. Physiol., 1908, **7**, 552–652). The possibility, which has not yet been finally answered, is that at some stage in the technique there is a shift in the position of chloride. The mere application of the silver reagent may conceivably withdraw chloride from the cell. For these reasons prior treatment of the tissue by the **Altmann-Gersh** freezing and drying method which reduces the chance of movement of chloride to a minimum is recommended.

1. Gersh (Gersh, I., Anat. Rec., 1938, **70**, 311–329) gives details of the proceedure on which the following instructions are based. Tissues frozen in liquid air, dried in vacuum, embedded in paraffin and sectioned at 15$\mu$ are mounted near

one edge on chemically clean large cover slips by simply pressing down with a finger, just melting over a flame and pressing down again. Immerse coverslips with attached sections in anhydrous petroleum ether (b.p. 20–40°C.) freshly distilled over sodium in a watch glass covered by another at all times except during actual manipulations. This removes the paraffin. Remove and burn off the ether quickly by a flame and allow to cool to room temperature. Then treat two coverslips with attached sections differently.

A. Cover for few seconds with drop of 60% aq. silver nitrate diluted with sufficient quantity of conc. phosphoric acid to prevent precipitation of rather large concentrations of phosphates and then saturate with silver chloride. After filtering 2–3 drops aq. dest. are added to every 10 cc. before using.

B. Cover similarly with: 60% aq. silver nitrate saturated with silver phosphate and silver chloride and dilute after filtering in the same way.

Decant fluids from both coverslips. Add to each 1 drop chemically pure glycerin and mount with section plus glycerin down on chemically clean slides. Expose both to carbon arc radiation for same length of time but at a distance not to warm the specimens. Examine immediately the reduced silver by direct illumination or in the dark field. A. shows specifically only the chloride and B. the same amount of chloride plus maximal concentrations of phosphate and some carbonate.

2. *Dichlorfluorescein method* (Bensley, R. D. and S. H., Anat. Rec., 1935, **64,** 41–49). For the lung of a rabbit. Inject 1% aq. dichlorfluorescein intravenously until the animal becomes quite yellow. Then kill it and inject 10% aq. silver nitrate or **Silver Citrate** solution either intratracheally or directly into the lung substance by a hypodermic syringe until the lung is moderately distended. In about 20 min. the color reaction reaches its maximum. The silver chloride becomes pink owing to adsorption of the dichlorfluorescein on the positively charged silver chloride molecule. Then fix pieces of lung in 10% neutral formalin and make frozen sections. Examine immediately for best color reaction. Dehydrate the sections, clear in absolute alcohol and iso-safrol and mount in balsam. The color reaction is not permanent but is masked and finally lost by the browning and blackening of the silver. It is not a true microchemical test; but it does detect the presence of chlorides though they are mobilized by the silver and tend to move to the periphery of the cell. The alveolar epithelial cells are outlined by pink stippling and their cytoplasm is also stippled and the nuclei are richly stippled. Mesothelial and endothelial cells are brilliantly and completely outlined in pink. The technique was first suggested by David M. Ritter.

The location of chloride is a matter of great importance. Lowry, O. H. and Hastings, A. B. in Cowdry's Problems of Ageing, Baltimore: Williams & Wilkins, 1942, 936 pp. cite the following as evidence for the extracellular position of chloride in skeletal muscle:

(1) Direct microscopic studies showing that chloride is exclusively extracellular (Gersh, I., Anat. Rec., 1938, **70,** 311–329).

(2) Perfusion experiments showing that chloride can be removed without apparently affecting the intracellular phase (Amberson, W. R. et al., Am. J. Physiol., 1938, **122,** 224–235).

(3) Variations in amount of chloride and in acid base balances of tissues can only be accounted for by assuming an an extracellular position for chloride (Hastings, A. B. and Eichelberger, L., J. Biol. Chem., 1937, **117,** 73–93).

(4) Isolated tissues equilibrated *in vivo* against solutions of varying chloride concentrations retain chloride in proportion to the concentration in the medium but at a very much lower level (Fenn, W. O., Cobb, D. M. and Marsh, B. S., Am. J. Physiol., 1934, **110,** 261–272; Eggleton, M. G. and P. and Hamilton, A. M., J. Physiol., 1937, **90,** 167–182).

(5) Conclusion that in many tissues for all practical purposes all radioactive sodium and radioactive chloride remain outside the cells (Manery, J. F. and Bale, W. F., Am. J. Physiol., 1941, **132,** 215–231; Manery, F. W. and Haege, L. F., *ibid,* **134,** 83–93).

See, however, Heilbrunn, L. V. and Hamilton, P. G., Physiol. Zool., 1942, **15,** 363–374 for demonstration of chloride in muscle fibers.

If chloride is always extracellular in all tissues it is possible accurately to measure the amount of extracellular fluid and a new chapter in histochemistry is opened. Lowry and Hastings give an example. If rat muscle is found to contain 10.5 milliequivalents of chloride per kilogram of tissue and the serum of the same animal 105.2 milliequivalents of chloride per kilogram of serum water, in view of the Donnan effect on chloride distribution it can be calculated that a kilogram of extracellular fluid contains 109.7 milliequivalents of chloride. Con-

sequently the sample of muscle contains $\frac{10.5}{109.7} \times 1000 = 96$ gms. of extracellular fluid per kilogram. When the extracellular fluid contains collagenic and elastic fibers, collagen and elastin must be determined and the necessary corrections made as well as for blood and fat when these are present. When the intracellular phase is chiefly composed of a single type of cell as in skeletal or cardiac muscle the further evaluation of intracellular components is not difficult. Taking every known precaution, evidence can apparently be collected of the relative composition of extracellular and intracellular phases.

**Chloroprene,** see **Neoprene.**

**Chlorothymols,** as preservatives of gelatin, glues, starches, etc. (Law, R. S., J. Soc. Chem. Ind., 1941, **60,** 66).

**Cholesterol** (esters) = cholesterides. In unstained frozen sections mounted in syrup of levulose they show no color of their own; but the **Liebermann-Burchardt Reaction** in frozen sections of formalin fixed tissue is positive. **Digitonine Reaction** in similar sections yields a complex in which the esters, if present, will color with **Sudan III** and lose birefringence in polarized light. See **Lipids** tabular analysis, see **Schultz** test for cholesterol and its esters.

**Cholesterols** (free). In unstained frozen section mounted in syrup of levulose, they show no color of their own. **Liebermann-Burchardt Reaction** in frozen sections of formalin fixed tissue is positive: blue, purple or violet then becoming green. **Digitonine Reaction** in similar sections yields strongly birefringent crystals and rosettes which do not stain with **Sudan III.** See **Lipids,** tabular analysis.

**Cholinesterase.** Important since it catalyses hydrolysis of acetylcholine to choline and acetic acid. Histological localization is difficult but Couteaux, R. and Nachmansohn, D., Proc. Soc. Exp. Biol. & Med., 1940, **43,** 177–181 found it present in parts of guinea pig's muscle that contained motor-end plates and absent in parts devoid of them. For recent data see Blaschko and Jacobson (Bourne, pp. 221–224). Sharp localization has been found in the giant nerve fiber of squids (Nachmansohn, D. and Steinbach, H. B., Science, 1942, **95,** 76–77).

**Chondriosomes,** see **Mitochondria.**

**Chondriotin Sulphuric Acid.** Present in cartilage and bone, stains metachromatically with basic dyes, described in detail by Lison, L., Arch. de biol., 1935, **46,** 599–668. See **Mucoproteins.**

**Chorioallantoic Membrane.** 1. Vital staining of virus lesions in membrane (Cooke, J. V. and Blattner, R. J., Proc. Soc. Exp. Biol. & Med., 1940, **43,** 255–256). Place 1 cc. 0.5% aq. trypan blue directly on membrane through window in shell. Rotate egg gently and return to incubator, 10–30 min. Small lesions require longer time to stain than large ones. Remove membrane, wash it gently in physiological saline and fix flat in 10% formalin, a few minutes. Make up glycerin jelly by soaking 5 gms. gelatin in 44 cc. aq. dest. Then add 50 cc. glycerin and 1 cc. phenol. Heat gently and stir. Flatten membrane on a 2 × 2.5 in. slide, warm glycerin jelly to about 70°C. Add drop by drop to membrane until well covered. Flame a cover glass and apply with slight pressure until it has begun to set. Remove hardened jelly around edges and seal with balsam. Foci of virus increase are sharply marked by clumps of deep blue stained cells.

2. Cultivation of microorganisms. The membrane has been shown to be an excellent medium for the cultivation of viruses by Goodpasture, E. W., Woodruff, A. M. and Buddingh, G. J., Am. J. Path., 1932, 8, 271–282 and many others. Its usefulness has been extended to Rickettsiae and spirochetes by Goodpasture, E. W., Am. J. Hyg., 1938, 28, 111–119, to fungi by Moore, M., Am. J. Path., 1941, 17, 103–125 and to acid-fast bacteria by Moore, M., Am. J. Path., 1942, 18, 827–847. This method of inoculation has the advantage over laboratory animal inoculation in that lesions will develop in the former within 5–8 days as compared to weeks or months in the latter; most organisms will produce definite and usually characteristic lesions in the chick membrane, whereas they may have no effect on experimental animals, often requiring human subjects; and because the lesions are so readily visible and traceable the chorioallantois serves well as a means of virulence determination.

The technique is essentially that of Goodpasture and Buddingh (E. W. and G. J., Am. J. Hyg., 1935, 21, 319–360) with some slight changes. Fertile eggs are incubated 12 days in an electrical thermostat-controlled incubator regulated to maintain a temperature of 98°F. The eggs are turned twice daily. A cm. square window is cut in the shell above the embryo, exposing the chorioallantoic membrane. The position of the embryo is determined by candling. The membrane is then inoculated directly with the fungus and the window is covered with a sterile coverslip and sealed with a paraffin-vaseline mixture (9 parts vaseline, 1 part paraffin). After inocu-

lation, the eggs are set in a bacteriologic incubator and maintained at a temperature of approximately 33°C., without turning. The membrane is watched daily through the window. When the inoculated area has shown marked change, the shell is cut below the window and the membrane exposed. The chorioallantois is cut with a pair of fine curved-end scissors, removed, fixed in Zenker's solution (with 5% glacial acetic). After washing, dehydrating, clearing in xylol, and imbedding in paraffin, it is sectioned and stained. Various staining techniques can be used depending on the organism inoculated. In general, for fungi, **Loeffler's** methylene blue and eosin have given satisfactory results.

**Chor's Modification** of Ranson's pyridine silver method was worked out in our laboratory to show alterations in motor end plates in biceps and triceps of monkeys in experimental poliomyelitis (Chor, H., Arch. Neurol. & Psychiat., 1933, **29**, 344–357). Fix in 1% ammonia water (28% Merck) in 95% alcohol for 24 hrs. Wash in aq. dest., ½ hr. Pyridine, 48 hrs. Wash in 8 changes aq. dest. during 24 hrs. 2% aq. silver nitrate in dark at room temperature, 72 hrs. Reduce 6–8 hrs. or over night in: pyrogallic acid, 4 gm.; aq. dest., 95 cc.; formalin, 5 cc. Dip in water and transfer immediately to 95% alcohol for a few seconds. Place tissue on slide with longitudinal markings of fibers visible. Add a second slide and squeeze gently. Trim edges with sharp knife so that neat, flat blocks result. 95% alcohol, 30 min. Absolute alcohol, 2 changes, over night. Xylol, 10–12 hrs. until blocks are clear. Imbed in paraffin 8 hrs. changing repeatedly each hr. for first five. Cut serial sections 10 $\mu$. Mount in neutral balsam. Nerves, dark brown or black; muscle and connective tissue, yellow.

**Chrom Blue GCB,** see **Gallocyanin.**

**Chromaffin Reaction** (chromic salts + L. *affinis*, akin). Brown coloration when treated with fixatives containing bichromate. In adrenal medulla adrenalin is revealed by this brown color but the reaction can also be elicitated by potassium iodate and is not altogether specific for adrenalin. Lison (p. 147) advises fixation in **Formol-Müller** or in 5% potassium iodate containing 10% of formol. After the usual fixations chromaffin substances can be demonstrated simply by treating the sections for a few hours with 3% aq. bichromate or iodate of potassium (Lison). See **Vulpian Reaction** and **Osmic Acid.**

**Chromatin Stains.** The most specific stain for basic chromatin is methyl green. Bismark brown is less so. Safranin is useful for chromatin if a red coloration is desired as in the safranin-light green combination. Tests for **Iron** and **Thymonucleic Acid** are listed separately. See **Idiochromatin, Linin, Chromosomes** and **Nucleolus.**

**Chromatophores.** These, when present in the dermis, are also called melanoblasts, see **Dopa Reaction** for their demonstration.

**Chrome Violet CG** (CI, 727). A carboxyl derivative of pararosolic acid.

**Chromic Acid** is purchased as the red crystals of chromic anhydride which dissolve easily in water forming chromic acid. The crystals should be kept in a bottle with closely fitting glass stopper because they are highly deliquescent. Alone in very dilute solution chromic acid is helpful in **Maceration.** When applied in aqueous solutions of about 1% to a slice of fresh adrenal it produces a brown color in the medulla known as the chromaffin reaction. In mixtures with other chemicals it was more used as a fixative 50 years ago than today but in **Perényi Fluid** it is recommended strongly by Lee (p. 32) for embryos, segmenting eggs, etc. It is also a component of Flemming's fluid.

**Chromidial Substance,** a designation often applied to basophilic cytoplasmic material supposed to be of nuclear origin and therefore to resemble the extranuclear chromatin (chromidia) of protozoa. It is nongerminal chromatin or trophochromidia in contrast to germinal or idiochromidia (G. *idios*, individual, one's own). See **Nissl bodies.**

**Chromolipoids.** In contrast to the carotinoids, which are hydrocarbons, the chromolipoids are fats or derivatives of fats themselves colored. They occur frequently especially in nerve cells, interstitial cells of the testicle and in the adrenal, and are easily distinguishable from carotinoids because they do not give the color reactions with sulphuric acid and iodine-iodide. From melanins they are to be distinguished by not dissolving in alkalies, by staining with sudan and scharlach and by not reducing ammoniacal silver nitrate. The following method of Hueck is given by Lison: Stain with nile blue. Treat the sections for 24 hrs. with aq. dest. oxygenated 3% (= commercial hydrogen peroxide diluted with 12 volumes water). This leaves the chromolipoids blue, the melanins decolorized. Lison concludes that distinction from pigments of hematogenous origin is not so easy because some chromolipoids contain iron. See **Lipids,** tabular analysis.

**Chromophil** (G. *chrōma*, color and *phileō*,

I love), a loose term applied to almost any granule, cell, or tissue which has a pronounced affinity for stains. Basophilic cytoplasmic materials in gland cells and in nerve cells (**Nissl bodies**) are sometimes called chromophil, moreover chromophil reaction is unwisely used to designate the **chromaffin reaction** of epinephrin producing tissues.

**Chrom-Osmic-Acetic** fixative, see **Lillie's.**

**Chromosomes,** chlorazol black E + acetocarmine (Nebel, B. R., Stain Techn., 1940, **15,** 69–72). Fixation in cold Flemming's fluid plus urea (Hance, R. T., Anat. Rec., 1917, **12,** 371–382). Microincineration of (Barigozzi, Cl., Bull. d'Hist. Appl., 1938, **15,** 213–219). Method of localization of genes by experimental deletions, distribution of protein and nucleic acid, classification, etc. (Painter, T. S., J. Roy. Micr. Soc., 1940, **60,** 161–176). Feulgen stain for chromosomes (Mensinkai, S. W., J. Roy. Micr. Soc., 1939, **59.** 82–112). Acetic-orcein is advocated as a new stain-fixative for chromosomes (LaCour, L., Stain Techn., 1941, **16,** 169–174).

**Chromotrope 2R** (CI, 29)—acid phloxine GR, chromotrope blue 2R, fast fuchsin G, XL carmoisine 6R—An acid mono-azo dye employed by Lendrum, A. C., J. Path. & Bact., 1935, **40,** 415–416 in a study of breast carcinoma and skin lesions as counterstain for celestin blue.

**Chromotrope Blue 2R,** see **Chromotrope 2R.**

**Chrysoidin Y** (CI, 20)—brown salt R, dark brown salt R—A basic mono-azo dye suggested by Conn (p. 46) as a substitute in some techniques for Bismark brown. Used as stain for mitochondria and Golgi apparatus viewed in polarized light (Monne, L., Protoplasma, 1939, **32,** 184–192).

**Chylomicrons** (lipomicrons). These tiny fatty droplets are easily demonstrated by dark field examination of blood of a person or animal fed butter or cream. The increase begins about 1 hr. there after and reaches a maximum at 4 hrs. after which the number of chylomicrons declines. By contrast a carbohydrate meal of rice and sugar or a protein meal of whites of boiled eggs and salt does not result in an increase. For details see Gage, S. H. and Fish, P. H., Am. J. Anat., 1924–25, **34,** 1–86; also, Hadjioloff, A., Bull. d'Hist. Appl. 1938, **15,** 81–98.

**Ciaccio,** methods for lipoids. One of the simplest is: Fix small pieces 2 days in: 5% potassium bichromate, 80 cc.; formalin, 30 cc., acetic acid, 5 cc. 3% potassium bichromate for 5–8 days. Running water 24 hrs. Ascending alcohols, 24 hrs. Abs. alcohol 2 hrs., xylol, 1 hr., xylol-paraffin at 60°C., 1 hr. Paraffin 1–1½ hrs. Pass sections down to 70% alcohol, stain ½–1 hr. at 30°C. in: 80% alcohol, 95 cc., acetone 5 cc. saturated at 50°C. with sudan III then cooled and filtered. Rinse in 50% alcohol, wash in water, counterstain with hemalum. Mount in syrup of Apathy (or glycerin). Lipoids yellow orange. Lison (p. 206) questions specificity for lipoids and gives in addition, with useful comments, several other methods of Ciaccio.

**Cilia.** The quickest method is to remove a piece of fresh ciliated epithelium from the respiratory nasal mucosa of an anesthetized or recently killed animal. Cut up finely with scissors, tease out small pieces with needles, mount in isotonic salt solution and examine at low magnification in the dark field. A simple technique of demonstrating the movement of cilia is to examine at a magnification of about 80 diameters the epithelium of the roof of a frog's mouth by reflected light. The angle between incident and reflected light should be about 90°. A strong source of illumination is required with a water screen to remove the heat. A useful set up is described and illustrated by Lucas (A. M., Arch. Otolaryng., 1933, **18,** 516–524). Many excellent moving pictures have been made of ciliary action of which one by Dr. Arthur Proetz is recommended for teaching purposes.

When cilia are present they can be seen in almost any properly stained section of well fixed material. A good stain for cilia is iron hematoxylin with suitable counterstain, after formalin-Zenker fixation. Engelman (Lee's Vade Mecum p. 509) found that ciliated cells of Lamellibranchs could be well isolated by maceration in 4% aq. potassium bichromate and in 0.1% osmic acid. This should be tried for mammalian ciliated cells. Cilia and their basal granules are often sharply blackened by silver impregnation. See Cowdry's description of flagellated thyroid cells of the dogfish (Cowdry, E. V., Anat. Rec., 1921, **22,** 289–299). Centrosomes and diplosomes are often revealed in ciliated cells particularly in those undergoing differentiation. See *Centrosomes.* Literature on ciliated epithelia is well presented by Lucas in Cowdry's Special Cytology, 1932, 409–474. See his illustrations. Application of technique of microdissection to ciliated cells is described by Worley, L. G., J. Cell. & Comp., 1941, 18, 187–198.

**Cinnamon Oil** (Cassia oil) resembles clove oil and is particularly recommended by Lee (p. 70) for clearing. Two kinds are given in Merck Index. The U.S.P.XI

variety contains 80–90% cinnamaldehyde.

**Citrate** of sodium can be used as an **anticoagulant** in the proportion of 18 cc. of 2% aq. sodium citrate to 100 cc. of blood.

**Clarite X** (Neville Co., Pittsburg) 60% in toluol is suggested as substitute for balsam owing to its neutral reaction, lack of yellow color and quickness of hardening. Clarite, also called Nevillite V, is useful if added to paraffin when one wishes to obtain thin sections when it is not convenient to imbed in a very high melting point paraffin. Wehrle, W., Stain Techn., 1942, **17**, 131–132 advises imbedding in a mixture of 90% paraffin (m.p. 53°C.), 5% bleached beeswax and 5% clarite and the elimination of electrical charge when ribbons are cut by a spark-coil device described by Blandau, R. J., Stain Techn., 1938, **13**, 139–141.

**Clark and Lubs Buffers** (Clark, W. M. The Determination of Hydrogen Ions, Baltimore: Williams & Wilkins, 1928, 717 pp.). Prepare: (1) A solution containing M/5 boric acid and M/5 potassium chloride made by dissolving 12.368 gms. of $H_3BO_3$ and 14.912 gms. of KCl in aq. dest., and diluting to 1 liter. (2) A M/5 sodium hydroxide (carbonate free) solution by dissolving 50 gms. of NaOH in 50 ml. (cc.). aq. dest. in a Pyrex flask. Let stand overnight to allow the sodium carbonate to settle, or filter through a Gooch or sintered glass crucible. (Exclude air to prevent formation of more carbonate by atmospheric $CO_2$.) Keep the strong alkaline solution in a paraffin-lined glass bottle. Dilute with aq. dest. which has been boiled to remove the excess $CO_2$ so that the solution is about 1 N. Then make an approximately M/5 solution of the alkali which can be accurately standardized against potassium acid phthalate.

To make buffer of the desired pH add to 50 cc. of (1) M/5 $H_3BO_3$-KCl the designated amount of (2) M/5 NaOH and dilute to 200 cc. with aq. dest. Or combine the two in similar proportions but in larger amounts to minimize error in measurement.

| pH | cc. of M/5 N |
|---|---|
| 7.8 | 2.61 |
| 8.0 | 3.97 |
| 8 2 | 5.90 |
| 8.4 | 8.50 |
| 8.6 | 12.00 |
| 8.8 | 16.30 |
| 9.0 | 21.30 |
| 9.2 | 26.70 |
| 9.4 | 32.00 |
| .6 | 36.85 |
| .8 | 40.80 |
| 0.0 | 43.90 |

**Cleaning Glassware.** Pulverize 20 gms. potassium bichromate. Dissolve this in 200 cc. aq. dest. with aid of a little heat. Add *slowly* 20 cc. sulphuric acid C.P. Before treating beakers, graduates, bottles, etc. with this acid cleaning solution first wash them in soap and hot water. Rinse in water to remove the soap. Leave in cleaning solution 2 hrs. or more. Rinse in running tap water and dry with opening downward on drying racks as in biochemical laboratories if possible in a dust free cupboard. For *new* slides and cover glasses wash in the same way and after final rinsing in tap water store in fresh 95% alcohol in covered dishes until they are required for use when they should be wiped with gauze. For *old* slides and cover glasses soak in xylol to permit separation and removal of most of balsam. Then leave in waste alcohol several days. Soak for a day or more in strong soap solution. Wash in running water. Clean in cleaning solution. Wash in water and store in 95% alcohol. Unless strict economy is necessary it is hardly worthwhile to use slides and covers twice especially when the former have been marked with diamond pencils.

**Clearing** is a process in microscopic technique which is required in three different situations.

1. *As the step following dehydration in paraffin imbedding.* The tissue becomes translucent but this is not the essential feature of the process. What is necessary is for the alcohol, which is not a paraffin solvent, to be removed by the clearing agent before the tissue is infiltrated with paraffin. Consequently the agents must mix freely with alcohol on the one hand and with paraffin on the other. Of them xylol is by far the most widely used and rightly so. Two changes of half absolute alcohol and xylol within 1 hr. and 2 changes of xylol within the next 3–4 hrs. are usually sufficient for slices of tissue 4–6 mm. thick, but the time should not be extended beyond that needed to attain translucency because so doing causes a hardening and a shrinkage of the tissue.

Several other substances can be used in place of xylol. *Cedar wood oil* is according to Lee (p. 80) the very best clearing agent for paraffin imbedding. It penetrates rapidly, does not make the tissues brittle, and, when not entirely displaced by paraffin, does not seriously interfere with sectioning. First treat the tissue with ½ absolute and xylol for about 2 hrs. The time required in the oil of cedarwood is however a little longer than in the case of xylol used alone, say 12 hrs. Some recommend 2 changes of

xylol (about 30 min.) after the oil of cedarwood before entering ½ paraffin and cedarwood oil.

*Methyl benzoate* is now quite popular. Pass the tissue from absolute alcohol through 2 changes of pure methyl benzoate within 12–24 hrs. When it has been definitely cleared remove benzoate by 2 changes of benzol (½–1 hr.) before passing into paraffin, or half benzol and paraffin.

*Chloroform* penetrates poorly and should not be employed unless called for. It has the further disadvantage that unless completely removed in the paraffin bath, it will make the final paraffin block soft and unfit for cutting. The usual practice is to clear very small pieces for about 12 hrs. in 2 changes, or as long as may be necessary to make them transparent, and in the imbedding to use 4 changes of paraffin.

A more rapid method is to pass directly from the fixative, Bouin or formalin, without washing, to 3 changes of pure *dioxan* within 4 hrs. and thence into 3 changes of paraffin as advised by Graupner, H. and Weissberger, A., Zool. Anz., 1931, **96**, 204–206. Stowell, R. E., Stain Techn., 1941, **16**, 67–83 confirms and extends earlier work of Seki which shows that, although xylol shrinks tissues more than dioxan, in placing in hot paraffin, the final shrinkage is greater in tissues after dioxan. When great haste is necessary Mallory (p. 54) suggests acetone ½–2 hrs.; benzol, 15–30 min.; and paraffin 3 changes, 30–90 min. The shrinkage, however, is very marked and it would probably be better to use **Frozen Sections.**

By the **Altmann-Gersh** technique, which is at once very time consuming and very valuable for special purposes, fixation, alcoholic dehydration and clearing can be side stepped and the dried tissue directly impregnated with paraffin.

2. *As the step following dehydration of sections before mounting.* The clearing is of course easier and much quicker owing to the thinness of the tissue. Again xylol comes first and will probably not be displaced though some prefer toluol. It is not necessary to protect against shrinkage and brittleness. When desired, abs. alc. can be omitted and the clearing be done from 90 or even 80% alc. with terpineol, clove oil, anilin oil, beechwood creosote, Bergamot or some other substance.

3. *As a means of rendering clearly visible certain structures in embryos or whole tissues.* Clearing is generally done by the **Spalteholz** method. See **Cartilaginous Skeleton** and **Ossification** centers. When glycerin mixtures are employed as **Mounting Media** they also clear the tissues.

**Cloudy Swelling.** This is a marked swelling and granulation of the cytoplasm of cells. It is sometimes observed post-mortem in acute febrile conditions especially in the kidneys, liver and myocardium. An almost meaningless synonym, often used, is **Parenchymatous Degeneration.** The extent of cloudy swelling that may occur *in vivo* and from which the cells may recover is not known. The fatty droplets present can be demonstrated in sudan stained frozen sections of formalin fixed material. Special stains for **Fibrin, Myofibrils** and **Mitochondria** may be desirable.

**Coacervates** (L. acervus, a cloud or swarm) are masses of particles clumped together (but encased in a little water) by a change in their electrical charge while in colloidal suspension in water or by dehydration with resultant loss of loosely bound water. Hirsch (G. C., Form und Stoffwechsel der Golgi-Körper. Protoplasma Monographs, Berlin, 1939) has likened the Golgi apparatus to a coacervate. See Bensley, R. R., Anat. Rec., 1937, **69**, 341–353 for critical consideration of suggestion that mitochondria are coacervates.

**Cobalt Nitrate Silver** for **Golgi Apparatus.**

**Cochineal** (CI, 1239) is a dye extracted from a tropical insect. It has been used mostly for staining *in toto* of small invertebrates. Mayer's alcoholic cochineal is a popular preparation made, according to Lee (p. 149), by powdering 5 gm. cochineal with 5 gm. calcium chloride and 5 gm. aluminum chloride to which 100 cc. 50% alcohol and 8 drops of nitric acid (sp. gr. 1.20) are added. Heat to boiling point, cool, shake occasionally during several days and filter. Before staining bring objects to 70% alcohol, destain if necessary in 70% alcohol containing 0.1% hydrochloric acid. Dehydrate, clear and mount in balsam. Nuclei are colored red and other structures a variety of colors from red to deep purple. In some respects it is better than carmine. Neither fade.

**Cochlea,** see **Ear.**

**Coelestin Blue,** see **Skyblue.**

**Coeline,** see **Skyblue.**

**Coeruleum,** see **Skyblue.**

**Colchiceine,** different from colchicine, see Ludford, R. J., Arch. f. exper. Zellf., 1935–36, **18**, 411–441.

**Colchicine,** see **Mitosis Counts.**

**Collagenic Fibers.** On boiling they yield collagen. They are also called white fibers in contrast to the elastic fibers which are distinctly yellow. Details

can be seen in fresh, unstained spreads of **Loose Connective Tissue.** The collagenic fibers are usually more numerous in subcutaneous connective tissue, less highly refractile than the elastic ones and of greater girth. They do not branch though the finer fibrils of which they are composed and which confer a faint longitudinal striation sometimes do. The R.C.A. electron microscope reveals a still finer system of collagenic fibrils (Scott, G. H. and Anderson, T. F., Anat. Rec., 1942, **82**, 445; Schmitt, F. O., Hall, C. E. and Jakus, M. H., J. Cell. and Comp. Physiol., 1942, 20, 11–33). On addition of dilute acetic acid they swell except at certain places in their length where they seem to be constricted by circular bands. The fact that they also easily pass from the gel to the sol state on alkalinization and when subjected to slight heat is the basis for methods of separating **Epidermis** from dermis.

The best stain for collagenic fibers in sections after Zenker fixation is anilin blue in **Mallory's Connective Tissue Stain** and in **Masson's Trichrome Stain. Phosphomolybdic Acid Hematoxylin** also gives a fine coloration of collagenic fibers. See **Van Gieson, Buzaglo.** The technique of microincineration as adapted to collagenic fibers is described by Allara, E., Bull. d'Hist. Appl., 1938, **15**, 220–242. See **Tendons.**

**Collodions.** There are several. See U.S.P. XI. All are solutions of **Pyroxylin.**

**Colloxylin,** see **Pyroxylin.**

**Colophonium,** usually dissolved in turpentine is employed to mount sections. Not advised.

**Color Preservation** in museum specimens. Fix 24 hrs. or less in 10% formalin. Wash in running water 3–6 hrs. Stand in 2% aq. ammonia 5–10 min. which hastens return of original colors. Running water another hour. Mount for permanent exhibition in mixture made as follows: Filter a sat. sol. antimony trioxide in aq. dest. (about 5 gm. per liter). To each 1000 cc. filtrate add 100 gm. potassium acetate, 100 gm. chloral hydrate and 50 cc. glycerin. Stir until completely dissolved (Meiller, F. H., J. Tech. Methods, 1938, **18**, 57–58).

Mallory (p. 380) recommends for this purpose the methods of Kaiserling and Jores.

There are 3 *Kaiserling* solutions:

1. *For fixation:* Formalin, 40 cc.; tap water, 2000 cc.; potassium nitrate, 30 gm. and potassium acetate, 60 gm. Small specimens require 1–14 days. Large ones can be more uniformly fixed by vascular **Perfusion.** Sometimes it is advisable to inject fixative into central parts of the tissue with a hypodermic syringe and long needle. Do not use too much pressure and be careful not to let any of the fixative spurt back into one's face. Before the next step wash in running water for about 12 hrs.

2. *For color restoration:* Place the tissue in 80% ethyl alcohol for 10–60 min. and watch for optimum coloration. If left too long in the alcohol the colors fade. Rinse in water and transfer to No. 3.

3. *For final preservation:* Change to glycerin, 500 cc.; 1% aq. arsenious acid, 200 cc.; tap water, 2300 cc.; potassium acetate, 250 gms.; thymol, 2.5 gm. To obviate difficulty of dissolving the arsenious acid and to sterilize add 25 gms. arsenic trioxide to 2500 cc. water + the thymol crystals first ground up in a mortar and place in steam sterilizer for 6 hrs. Then add other substances.

There are 2 *Jores* solutions.

1. *For fixation:* Chloral hydrate, 50 gms.; artificial Carlsbad salts (sodium sulfate, 22 gm.; sodium bicarbonate, 20 gm.; sodium chloride, 18 gm.; potassium nitrate, 38 gm.; potassium sulphate, 2 gm.), 50 gm.; formalin, 100 cc.; tap water, 1000 cc. Allow to act 2–14 days depending on size, wash 12 hrs. in running water.

2. *For final preservation:* Potassium acetate, 300 gm., glycerin, 600 cc.; aq. dest., 1000 cc.

Mallory suggests fixation in Jores' first solution and preservation in Kaiserling's third solution.

**Concentration.** 1. *Tubercle bacilli* in sputum. Nagy (A.H., J. Lab. & Clin. Med., 1939, **25**) having critically evaluated several techniques recommends *Pottenger's Dilution-Flotation method.* Shake equal parts sputum and 0.5% aq. sodium hydroxide for 10 min. Digest in water bath at 56°C. for 30 min. Add 1 ml. (=1 cc.) hydrocarbon (gasoline or xylene), then 200 ml. aq. dest. and shake 10 min. Allow hydrocarbon to collect at top 15–20 min. Take up hydrocarbon layer in rubber bulbed pipette. Keep in vertical position until supernatant fluid separates from hydrocarbon, 5–10 min. Make smears from hydrocarbon and dry. Remove hydrocarbon by washing with ether. Stain with carbol fuchsin 3 hrs. or longer. Decolorize with acid alcohol 30 sec. or less. If further decolorization is required employ 10% aq. sodium sulphate. Counterstain with 1% aq. picric acid or with methylene blue. The concentration of bacilli is about 33 times. Perhaps a modification of the method could be used for leprosy organisms in emul-

sions of tissues. See also Pottenger, J. E., Am. Rec. Tuberc., 1939, **40**, 581. Concentration of tubercle bacilli in spinal fluids (Hanks, J. H. and Feldman, H. A., J. Lab. & Clin. Med., 1939, **25**, 886–892). It is often necessary to concentrate for microscopic study objects which are not present in abundance and which might otherwise be overlooked. See examination of **Feces** for ova of parasites, of **Urine** for sediment.

2. *Leprosy bacilli* for chemical analysis. Ravold's method for leprosy bacilli can perhaps be used for others. Relatively large masses of bacilli-laden cells are dissected away from neighboring uninvolved tissue and from necrotic tissue when present in the centers of the nodules. They are placed in a Wueller press without addition of any fluid. On exertion of pressure many of the cells are ruptured and the tissue fluid, together with cytoplasm, nucleoplasm and some entire cells, passes through minute holes in the press and is collected, leaving most of the fibrous elements behind. Then a little saline solution is added and the material is ground up in sand and made up to a volume of about 50 cc. The sand is allowed to sediment out at the bottom of a centrifuge tube. The supernatant fluid is then centrifuged at low speed (300 r.p.m.). This throws all the rest of the debris to the bottom while the bacilli remain in suspension. The supernatant fluid, containing the bacilli, is again decanted and centrifuged at high speed (3500 r.p.m.) in an angle centrifuge for 1 hr. The supernatant fluid is discarded and the pasty material at the bottom of the tube, made up of bacilli, is diluted and washed by repeated centrifugation in some experiments with saline solution and in others with distilled water.

Beginning with a large nodule or with several small ones it is a simple matter to collect in 4 or 5 hrs. billions of bacilli. The pasty bacterial mass can be desiccated and weighed in grams. For our experiments we used only the wet bacilli. When viewed *en masse* they appear dense white with a faint shade of gray. They are not yellow or even yellowish. Examination of a thick smear, made after washing in saline, shows myriads of bacilli without any trace of cellular material. The bacilli retain to a remarkable degree their characteristic morphology, as seen in sections and in smears of fresh tissue, and their acid-fast properties are not interfered with. After washing in distilled water until the supernatant fluid gave no precipitate when added to an aqueous solution of silver nitrate, the bacilli do not fuse together but still remain discrete bodies though their shape is different. (Cowdry, E. V., Ravold, A. and Packer, D. M. Proc. Soc. Exp. Biol. & Med., 1939, **41**, 341–345).

**Congo Blue 3B,** see **Trypan Blue.**

**Congo Corinth G** or **GW,** see **Erie Garnet B.**

**Congo Red** (CI, 370). Synonyms: Congo, cotton red, A, B or C, direct red C, R or Y. An acid dis-azo dye which is an excellent indicator and a useful stain. Matsuura, S., Fol. Anat. Jap., 1925, **3**, 107–110 has obtained very fine coloration of the skin which he has illustrated in colors. Congo red is the basis of **Krajian's** stain for elastic fibers. See also Blackman, V. H., New Phytol., 1905, **4**, 173–174 (uredineae); Merton, H., Arch. Protistenk., 1932, **76**, 171–187; Cumley, R. W., Stain Techn., 1935, **10**, 53–56 (negative stain for bacteria), etc.

**Connective System.** Provides both for the binding together of parts and for their separation one from another by capsules, membranes and other structures (see Cowdry, p. 429–466). It ranges all the way from **Loose Connective Tissue** and **Fatty Tissue** through **Fibrous Connective Tissue** and **Tendons** to **Cartilage** and **Bone. Neuroglia** is a special form of it. In general there are three components, **Fibroblasts, Fibers** and **Tissue Fluid** (ground substance). Cells of hematogenous and lymphatic origin may be present since blood vessels and lymphatics run in connective tissue pathways. See techniques under these headings, also **Masson's Trichrome Stain, Mallory's Connective Tissue Stain, Phosphomolybdic Acid Hematoxylin, Van Gieson, Buzaglo,** etc.

**Connective Tissue Cells,** preservation of trypan blue and neutral red in those of loose connective tissue. Inject subcutaneously 5 cc. fresh sterile 1% aq. vital trypan blue (Coleman and Bell Co.) into a mature white rat weighing about 90 gms. and wait 48 hrs. Make up 0.02% certified neutral red (National Aniline in 0.9% NaCl). After slight etherization exsanguinate the animal. Inject neutral red into subcutaneous tissue of groin in several places near original puncture. After 3–5 min. remove small blobs of edematous tissue. Tease these out on clean slides with aid of needles and filter paper. When corners are dry spread is ready for direct observation under cover glass or for fixation. Make up 10% formalin. Test it by addition of a drop or two of neutral red. If this turns orange add a little N/10 HCl until it becomes red.

Fix in this formalin over night or for several days. Rinse in aq. dest. Counterstain in 1% fast green F.C.F. (Warner-Jenkinson Co.) in 2% aq. acetic acid for ½–1 min., pass through successive changes dioxan, 3–5 min. each. Agitate slightly. Mount in dioxan employing medium hardened diaphane (Will Corp.), redissolve in dioxan or pass through xylol and mount in balsam. Avoid alcohols. Note blue granules in macrophages and fine red granules in mast cells (Snook, T., Stain Techn., 1939, **14**, 139–142). See **Connective System.**

**Contraction Bands,** or waves, demonstration of in smooth muscle. Remove intestine of freshly killed cat, expose to air of room or rub with blunt end of scalpel. When preparations are made numerous contraction bands will be seen. Contrast with this intestine of cat killed with chloroform and not excised until rigor mortis begins in which muscle fibers are fully extended (Dahlgren, McClung, p. 430).

**Copper.** 1. *Microchemical tests.* Fix in formalin or alcohol, use same hematoxylin or methylene blue stain as for **Lead.** With former copper hemofuscin is blue and hemosiderin (iron pigment) is black, while with latter copper pigment is pale blue and the iron pigment uncolored (Mallory, F. B. and Parker, F., Jr., Am. J. Path., 1939, **15**, 517–522). See also Okamoto, K., Utamura, M. and Mikami, G., Acta Sch. Med., Univ. Imp. in Kioto., 1939, **22**, 335–360 (illustrated in colors); Mendel, L. B. and Bradley, H. C., Am. J. Physiol., 1905, **14**, 313–327 (bromine test for); Claude, A., Cold Spring Harbor Symposia on Quantitative Biology, 1941, **9**, 263–270 (copper of respiratory pigment); Hoagland, C. L. et al., J. Exper. Med., 1942, **76**, 163–173 (copper and other substances in virus elementary bodies).

2. *As vital stain.* Intravenous injections of colloidal solutions of copper in rabbits are described by Duhamel, B. G., C. rend. Soc. de Biol., 1919, **82**, 724–726.

**Copper Chrome Hematoxylin** (Bensley's) for mitochondria. Fix very small pieces in **Altmann's Fluid** or in **Acetic-Osmic-Bichromate** fixative of Bensley 12–24 hrs. Wash, dehydrate, clear, imbed in paraffin and cut sections at 4 or 5 microns. Deparaffinize. Sat. aq. copper acetate, 5 min. Several changes aq. dest., 1 min. 0.5% aq. hematoxylin, 1 min. After rinsing in aq. dest. pass to 5% aq. neutral potassium chromate, 1 min. which should turn sections dark blue-black. If they are only light blue, rinse in aq. dest. again place in copper acetate and repeat if necessary several times until no increase in color is obtained. Wash in aq. dest. and treat for few sec. with copper acetate. Wash in aq. dest. and differentiate under the microscope in Weigert's borax-ferricyanide mixture (borax, 1 gm.; potassium ferricyanide, 1.25 gm.; aq. dest. 100 cc.) diluted with twice the volume aq. dest. Wash in tap water, 6–8 hrs. Dehydrate, clear and mount in balsam. The mitochondria appear a beautiful deep blue against a yellowish background. It is important to have good, ripe hematoxylin. It is usually made by dilution from a 10% sol. in abs. alcohol. This method of staining should be tried after fixation in Regaud's fluid.

**Coproporphyrin** of megaloblasts in embryos, see **Porphyrins.**

**Coreine 2R,** see **Celestin Blue B.**

**Corinth Brown G,** see **Erie Garnet B.**

**Corn Blue B,** see **Victoria Blue R.**

**Corn Blue BN,** see **Victoria Blue B.**

**Cornyebacterium Diphtheriae.** Evaluation of methods for staining metachromatic granules (Morton, H. E., Stain Techn., 1942, **17**, 27–29).

**Coronary Arteries.** Their distribution may be demonstrated by injection of the easily recognizable fluids listed under **Blood Vessels.** Owing however to their great importance it is well to mention two special adaptations of the said fluids. Gross (L., The Blood Supply of the Heart in its Anatomical and Clinical Aspects. New York: Hoeber, 1921) employed injections of barium sulphate suspensions in gelatin followed by x-ray photographs; while Spalteholz (W., Die Arterien der Herzwand, etc., Leipsig: Herzel, 1924) used injections of cinnabar and other pigments likewise in gelatin which were later cleared by his method. Ehrich, Chapelle and Cohn (W., C., and A. E., Am. J. Anat., 1931, **49**, 241–282) found the latter technique preferable. **Celloidin** injections also give good results. Histological demonstration of the blood supply of the coronaries is described under **Vasa Vasorum.**

**Corpora Amylacea,** see **Prostate.**

**Corrosion Preparations.** In them the structures to be demonstrated are left while all the surrounding tissue is corroded and washed away, for instance **Celloidin** and **Neoprene** injections.

**Corrosive Sublimate,** see **Mercuric Chloride.**

**Corti,** organ of, see **Ear.**

**Cortin** (interrenalin), hormone of adrenal cortex.

**Cotton Blue,** see **Anilin Blue, Methyl Blue.** See **Fungi.**

**Cotton Corinth G,** see **Erie Garnet B.**

**Cotton Red,** see **Safranin O.**

**Cotton Red, A, B,** or **C,** see **Congo Red.**

**Cotton Red 4B,** see **Benzopurpurin 4B.**

**Cover Glasses,** see **Cleaning.**

**Creosote** (Beechwood) is an important clearing agent for celloidin sections. It is a mixture of phenols, mainly guaiacol and creosol.

**Cresyl Blue 2RN,** or **BBS,** see **Brilliant Cresyl Blue.**

**Cresyl Violet**—cresylecht violet (cresyl fast violet)—Commission Certified. A basic oxazin dye. A technique for its use (or that of toluidin blue) in studies on the cytoarchitectonics of the nervous system is proposed by Landau, E., Bull. d'Hist. Appl., 1934, **11,** 44–46. As a stain for nerve cells in celloidin sections (Tress, G., and M., Stain Tech., 1935, **10,** 105–106). Wash low viscosity nitrocellulose (celloidin) sections of 10% formalin fixed tissues in aq. dest. Stain for 30 min. at 50°C. in cresyl violet, 0.5 gm.; aq. dest., 100 cc.; glacial acetic acid, 4 drops (filtered before using). Wash in aq. dest. Differentiate in 70% alcohol until most of stain leaves celloidin. Completely immerse for 2–5 min. in: chloroform, 60 cc.; abs. alc., 10 cc.; and ether, 10 cc. Almost no destaining of cells occurs but stain is removed from background. Differentiate in 100 cc. 95% alc. + 4 drops 1% aq. hydrochloric acid but stop while cells are a little darker than desired. Neutralize sections in 90% alcohol + a little sodium bicarbonate. Wash in 95% alcohol to remove the bicarbonate. Complete dehydration in 2 changes *n* butyl alcohol. Clear in 4 changes xylol and mount. See **Kallichrom.** *Note:* There are two different dyes sold as cresyl violet: (1) The C.C. product (Nat. Anilin, Mfgrs.; see Conn, 1940, p. 93) which is good in biopsy work; (2) The Grübler product (also sold by Coleman and Bell, but not on the market during the war) which is needed in neurological work, c.f. Tress, above.

**Cresylecht Violet,** intensification of metachromatic properties (Williams, B. G. R., J. Lab. & Clin. Med., 1934–35, **20,** 1185–1187).

**Croceine Scarlet,** see **Biebrich Scarlet,** water soluble.

**Crossman's** modification of Mallory's connective tissue stain (Crossman, G., Anat. Rec., 1937, **69,** 33–38). Deparaffinize sections of Zenker fixed material. Lugol's iodine, 5 min. Rinse in 70% alcohol, several changes. Wash 10 min. in running water. Overstain nuclei in Mayer's acid **Hemalum** or Weigert's **Iron Hematoxylin.** Wash in running water 10 min. Stain for 1 min. or more in: acid fuchsin (C.C.), 1 gm.; orange G (C.C.), 0.4 gm.; aq. dest., 300 cc.; thymol, 0.2 gm.; glacial acetic acid, 3 cc. Rinse in aq. dest. Decolorize in fresh 1% aq. phosphotungstic or phosphomolybdic acid until arterial media is red and adventitia is colorless. Rinse very quickly in aq. dest. Counterstain in 2% aq. anilin blue, W.S. (C.C.) 100 cc. + glacial acetic acid, 2 cc. or in 1% aq. light green SF yellowish (C.C.) 100 cc. + glacial acetic acid, 1 cc. Rinse in aq. dest. Decolorize in 1% acetic acid under microscope. Rinse in aq. dest. Dehydrate in 3 changes abs. alc. Clear in 3 changes xylol and mount. Like original method but nuclei brown or black and collagen blue or green depending on counterstain.

**Cryostat,** see **Altmann-Gersh** freezing and drying technique.

**Cryptococcus Hominis,** see **Blastomycosis.**

**Crystal Violet** as vital stain for fibroblast nuclei (Bank, O. and Kleinzeller, A., Arch. exp. Zellforsch., 1938, **21,** 394–399). See **Anilin Crystal Violet** and **Gentian Violet.**

**Crystal Violet-Acid Fuchsin.** This is one of R. R. Bensley's neutral stains especially advocated for the demonstration of secretion antecedents in gland cells. The technique is described by the Bensleys (p. 97). To make stain add filtered sat. aq. acid fuchsin to similar solution crystal violet until precipitation is complete. Collect ppt. on filter paper, wash through once with aq. dest. Dry and dissolve in absolute alcohol to saturation. For staining add 5 cc. of above stock solution to 45 cc. 20% alcohol made from absolute. In this color paraffin sections of **Formalin-Zenker** fixed material for 5 min. Blot with filter paper in one hand and add with other hand absolute alcohol from a pipette, flood with absolute. Blot immediately. Add few drops clove oil. When differentiation, observed under microscope, is optimum transfer to pure benzol and mount in balsam.

**Crystal Violet and Alizarin,** see **Benda's Method** for Mitochondria.

**Curettings,** gelatin method for rapid frozen sections of (Meeker, L. H., J. Techn. Meth. & Bull. Int. Assoc. Med. Museums, 1936, **16,** 41–42).

**Cyanosine,** see **Phloxine B.**

**Cytocentrum,** centrosome plus centrosphere.

**Cytochrome.** This is the name given by Keilin (D., Proc. Roy. Soc., 1925, B, 98, 312–339) to hemin compounds of a reddish color which occur in oxidized or reduced condition in almost all living cells. Blaschko and Jacobson (Bourne, p. 192) have summarized our knowledge about them. They say that the red color of cytochrome can be observed

when a slice of brain tissue, from which the blood has been carefully washed out, is suitably illuminated by transmitted light. A thick suspension of yeast and the thoracic muscles of insects are also recommended as material. There are 4 cytochromes: a, b, c and $a_3$ recognizable spectroscopically. Cytochrome is oxidized by cytochrome—oxidase which is identical with indophenol oxidase and Warburg's respiratory enzyme. See study of cytochrome oxidase-cytochrome system in kidney (Flexner, L. B., J. Biol. Chem., 1939, **131**, 703–711). See **Oxidase.**

**Cytoplasmic Inclusions** caused by viruses. They are more diversified in size, shape and chemical composition than the **Nuclear Inclusions.** Frequently, as in the case of large **Negri Bodies,** they contain both acidophilic and basophilic components (**Trachoma Bodies**). Glycogen tests for Trachoma inclusion bodies are described by Thygeson, P., Am. J. Path., 1938, **14**, 455–462. The techniques mentioned for **Nuclear Inclusions** may be employed. See description by Goodpasture, E. W. and Woodruff, A. M., Am. J. Path., 1930, **6**, 699–711; 713–720 of the reactions of fowl-pox inclusions to potassium hydroxide and other chemicals and the nature of the particles. See also **Borrel, Guarnieri** and **Kurloff** bodies. **Rickettsia** are not to be listed as cytoplasmic inclusions but Giemsa's stain is the best for them.

**Dahlia,** see **Hofmann's Violet.**

**Dahlia B,** see **Methyl Violet.**

**Damar** is gum damar dissolved in xylol and used to mount sections.

**Dark Brown Salt R,** see **Chrysoidin Y.**

**Darkfield Microscope.** This is constructed on the same principle as that of the *ultramicroscope* developed more than a generation ago by Siedentopf and Zsigmondy in so far that it depends on the Faraday-Tyndall phenomenon of the illumination of minute particles by light reflected from their surfaces as when tobacco smoke drifts into a beam of light in an otherwise darkened room. In the old ultramicroscope (intended mainly for colloidal suspensions) the illumination was from one side through a slit, while in the modern darkfield condenser (designed for work with cells) it is from below at the sides. Ordinary oculars and low power objectives can be employed but for oil immersion work the best objective is a 3 mm. fitted with an iris diaphram. Especially adapted and more powerful objectives can usually be obtained and are of great value. Examination in the darkfield is required for the study of **Microincineration** preparations, of living **Spirochetes** and other small microorganisms, of **Chylomicrons** and a wide variety of cellular components. Ordinarily the full usefulness of the method is not realized because investigators content themselves with inadequate light and dry, low power, objectives.

**Davenport's** method for staining nerve fibers in paraffin sections with protargol in 2 hours (Davenport, H. A., McArthur, J. and Bruesch, S. R., Stain Techn., 1939, **14**, 21–26). For background see earlier papers by Davenport and his associates also general account of **Silver Methods.** Fix small nerves in large tissue blocks over night in: pure formic acid, 5 cc.; trichloracetic acid, 5 gm.; *n* propyl alcohol, 25 cc.; *n* butyl alcohol, 65 cc. (freshly made up). Transfer to 95% ethyl alcohol and through several concentrations (1–2 hrs. in each) to water. Then dehydrate, imbed in paraffin and section in usual way. Remove paraffin and impregnate in 10% aq. silver nitrate (Merck's C.P.) for 1 hr. at 58–62°C. Rinse in 3 changes aq. dest. about 30 sec. in each. Impregnate at room temperature 1 hr. in 0.2% protargol (Winthrop Chemical Co.). Rinse for 2 sec. in aq. dest. and reduce 1 min. in 5 parts A and 1 of B. A = sodium sulfite, 10 gm.; aq. dest., 90 cc. B = sodium (or potassium) bisulfite, 5 gm.; amidol, 1 gm.; aq. dest., 95 cc. Wash in running tap water several minutes and change to aq. dest. (1 change). Tone until all yellow is replaced by gray (several min.) in 0.1% aq. gold chloride. Repeat washing in tap water and aq. dest. Reduce by dropping 0.5–1% aq. amidol on slide few seconds. Wash, dehydrate, clear and mount. Amidol is diaminophenol hydrochloride in use as a photographic developer. For more details consult original paper.

**Dead Bacteria.** To distinguish from living try:

1. *Proca-Kayser stain* (Gay, F. P. and Clark, A. R., J. Bact., 1934, **27**, 175–189). Fix bacterial smear by drying and flaming. Stain 3–5 min. in Loeffler's alkaline methylene blue. Wash quickly and stain in Ziehl-Neelsen's carbol fuchsin only 5–10 sec. Wash and dry. Living bacteria blue, dead ones purple to red.

2. *Neutral red* (Knaysi, G., J. Bact., 1935, **30**, 193–206). Add a little neutral red to the medium. *Escherichia coli* and *Schizosaccharomyces pombi* are considered dead when tinged even slightly by the stain.

3. *Decolorization* (Prudhomme, R. O., Ann. Institut Pasteur, 1938, **61**, 512–518). Living bacilli separated from all tissue decolorize solutions of 1-naphthol-2-sodium sulphonate-indo-2-6-dibromphenol, O-cresol-2-6 dichlorophenol and O-chlorophenol-indo-2-6-dichlorophenol. Bacilli killed by 100°C. for 15 min. do not decolorize them.

The value of these methods is questionable.

**Dead Cells.** Often it is very difficult to say whether a particular cell was dead or alive when the preparation was made. The appearance of nuclei in **Postmortem Degeneration** may be a clue. Evans and Schulemann (H. M. and W. Science, 1914, **39**, 443–454) remarked upon the extraordinary rapidity with which dead cells take in vital benzidine dyes and the diffuse, uniform coloration that ensues. In cells supravitally stained with neutral red Lewis and McCoy (W. H. and C. C., Johns Hopkins Hosp. Bull., 1922, **33**, 284–293) employed the following criteria for death: "(1) loss of color from the granules and vacuoles; (2) diffuse pink staining of the cytoplasm and nucleus; (3) the appearance of a sharp and distinct nuclear membrane and a change in texture of the cytoplasm and nucleus." Using dark-field illumination W. H. Lewis (Anat. Rec., 1923, **26**, 15–29) observed the appearance in dying cells of certain very small brightly shining (white) bodies which he called *d* or "death granules." These were first in Brownian movement which soon ceased. To quote Lewis: "During the period when the cells were dying, spherical blebs often appeared on both the flat and rounded cells. These were pale grayish sacs with very thin walls and fluid contents in which varying numbers of small white granules in active Brownian motion were seen. The blebs varied in size and were occasionally as large as a contracted cell. Sometimes the blebs were so crowded with granules that they were milky in appearance. Frequently one would burst, freeing its granular contents into the surrounding fluid medium where they showed Brownian motion until they settled down on the slide." Luyet's (B., Science, 1937, **85**, 106) method for the differential staining of living and dead plant cells may prove of value for animal cells also. See **Necrosis, Necrobiosis.**

**Decalcification.** The removal of calcium so that bony tissues can be cut in sections. There are many methods almost all of which involve acid treatment. It is generally better to apply the decalcifying agent after fixation, particularly so when the agent is a poor fixative. The volume of decalcifier should be about 100 times that of the tissue. The usual, crude, way of testing the progress of decalcification is to stick a fine needle into the bone being careful to avoid the area that will be cut in sections; but less objectionable methods can be used, see **Teeth, Decalcification.**

Saturated aq. sulphurous, 5% trichlorlactic, 5% hydrochloric and equal parts of 1% hydrochloric and 1% chromic acids are all fairly good decalcifiers. Lactic, acetic, phosphoric and picric acids are usually unsatisfactory. Shipley (McClung, p. 347) recommends slow decalcification by long immersion in **Muller's Fluid** through liberation of small amounts of chromic acid from the bichromate. The bones of an adult rat require 21–30 days. The process can be hurried somewhat by using an incubator at 37°C. Adequate decalcification is detected by slight bending of the bone or by the needle method. Over decalcification is not likely.

For rapid decalcification he advises using sat. aq. phloroglucin to which 5–30% **Nitric Acid** is added. A somewhat slower formula is: nitric acid, 5 cc.; phloroglucin, 70 cc.; 95% alc., 1 cc.; and aq. dest., 30 cc. The phloroglucin allows use of stronger acids. 1–2% aq. hydrochloric acid decalcifies quickly but it causes the tissue to swell. **Formic Acid** 1–5% in 70% alcohol is, according to Shipley, the best decalcifying agent for large masses of bone. With 5%, the decalcification is completed in 4–5 days. Use 70% alc. not water, to wash out the acid.

Kramer and Shipley devised a **Magnesium Citrate** method of decalcification in neutral solutions. To make the decalcifier dissolve 80 gms. citric acid in 100 cc. hot aq. dest., add 4 gms. magnesium oxide and stir until completely dissolved. If the magnesium oxide contains carbonate it should be freshly ignited. Cool and add 100 cc. ammonium hydroxide (density 0.90) and aq. dest. to make 300 cc. Allow to stand 24 hrs. and filter. Titrate filtrate with hydrochloric acid to about pH 7.0–7.6 and add equal volume aq. dest. In decalcifying, this reagent should be changed every 3 days. A dog's rib is decalcified in approximately 15 days.

After decalcification, by whatever method, the bone, or the area of calcification, must be thoroughly washed to remove the decalcifer and imbedded in paraffin or celloidin. Some investigators prefer the latter but celloidin

sections are not so easily handled. See **Bones, Teeth.**

**Degeneration.** Because the structural organization of various sorts of cells is, like their function, so very different the types of degeneration leading to death are also different at least in many of their aspects. See **Nerve Fiber Degeneration, Cloudy Swelling, Necrosis, Caseation, Parenchymatous Degeneration, Postmortem Changes.**

**Dehydration** is the removal of water from a tissue preliminary to clearing and paraffin or celloidin imbedding. This is routinely done by treating the tissue after **Fixation** and **Washing** by passing it through a series of ethyl alcohols of increasing concentration. Usually the percentages are 30, 50, 70, 80, 95 and absolute. The time depends upon the size and kind of the tissue and the sort of fixative. For slices of tissue less than 3 mm. thick the dehydration can be accomplished in 6–12 hours. The alcohols for large slices fixed say in Zenker's fluid are ordinarily changed every morning and evening, but it is not desirable to leave them in absolute alcohol very long because it makes them brittle. Three to 6 hours should be sufficient. Tissues fixed in alcoholic solutions take a shorter time to dehydrate. After fixation in alcohol-formalin or in Carnoy's fluid the tissue can be dehydrated and partly washed in several changes of absolute alcohol skipping the lower grades of alcohol entirely.

When, for some reason, it is desired to eliminate treatment with absolute alcohol the tissues can be passed directly from 95% alcohol into **Aniline Oil** (say 30 min.) which is itself later removed, at least partly, in 5–10 minutes by washing in 2 changes of chloroform. Clearing is continued in chloroform for imbedding in paraffin, or the tissue may be passed from 95% alcohol, even from 80%, into **Terpineol** and cleared in half terpineol and xylol. Still another way to avoid absolute alcohol is to transfer from 95% alcohol to **Bergamot Oil** which serves as a clearing agent.

Several substitutes for ethyl alcohol as a dehydrating agent are available. Acetone is the best known. Dioxan will not only take the place of the alcohol but also that of the clearing agent so that it is possible to greatly simplify the technique and make the sequence: fixative to dioxan to paraffin. See **Dioxan** and note as to possible danger to those using it. Cellosolve has also been proposed as a dehydrating agent. Lee (p. 64) says that it is expensive, inflammable and quickly takes up water from the air. Whether it is injurious when breathed remains to be determined. On the whole it appears that little is to be gained by such substitutes. If alcohol must be avoided it is always possible to fix in formalin and to use frozen sections. By the **Altmann-Gersh** technique the tissues are dehydrated *in vacuo* while still frozen.

**Dehydropyridines.** Warburg noted a marked whitish fluorescence in ultraviolet light. Blaschko and Jacobson (Bourne, p. 196) report that the pyridines do not show this fluorescence and that the small granules that exhibit it in sections of living liver tissue may well be dehydropyridines. Their brilliant white fluorescence quickly fades.

**Delafield's Alum Hematoxylin.** To 400 cc. sat. aq. ammonia alum add 4 gms. hematoxylin dissolved in 25 cc. 95% alc. Leave exposed to air and light 4 days. Add 100 cc. methyl alc. and 10 cc. glycerin; filter. Filtrate will slowly ripen. To hasten ripening add 10 cc. hydrogen peroxide.

**Delta Dye Indicator,** see **Nitrazine.**

**Dental Enamel,** see **Enamel.**

**Dentin.** Can be studied in ground sections of undecalcified teeth as well as in paraffin and celloidin sections of decalcified ones (see **Teeth**). For the latter **Hematoxylin** and **Eosin, Mallory's Connective Tissue** stain and many others can be applied as in the case of decalcified bone. Hanazawa's (Dent. Cosmos, 1917, **59**, 125) methods for the minute structure of dentin are given in detail by Wellings, A. W., Practical Microscopy of the Teeth and Associated Parts, London: John Bale, Sons & Curnow. Ltd., 1938, 281 pp. Dentin can be advantageously examined after vital staining with **Alizarin Red S.** Its pH can be estimated (Grossman, L. I., J. Dent. Res., 1940, **19**, 171–172). For determination of rate of mineral replacement see **Radioactive Phosphorus**; for Korff's fibers, see **Teeth, Developing**; and for nerve endings, see **Teeth, Innervation.**

**Destin's** fixative. 1% aq. chromic acid, 99 cc.; formalin, 6 cc.; glacial acetic acid, 2 cc. After standing for a few days it becomes green when it can be used.

**Diacetin** (glycerol diacetate) use in flattening paraffin sections (Carleton, H. M. and Leach, E. H., J. Path. & Bact., 1939, **49**, 572–576).

**Diamin Red 4B,** see **Benzopurpurin 4B.**

**Diamine Bordeaux CGN,** see **Erie Garnet B.**

**Di-Amino Tri-Phenyl Methane Dyes.** Examples: brilliant green, fast green FCF, light green SF yellowish and malachite green.

**Diamond Green,** see **Brilliant Green.**

**Diamond Green B, BX** or **P Extra,** see **Malachite Green.**

**Dianil Blue H3G,** see **Trypan Blue.**

**Dianil Blue 2R** (CI, 265)—benzo new blue 2B, direct steel blue BB, naphthamine brilliant blue 2R—Conn (p. 63) gives the same formula for this acid dis-azo dye as that supplied by Corner, G. W. and Hurni, F. H., Am. J. Physiol., 1918, **46,** 483–486 and Sutter, M., Anat. Rec., 1916, **16,** 164–165 for dye employed by them in study respectively of corpora lutea and mammary glands but these authors do not employ the name: dianil blue.

**Dianil Red 4B,** see **Benzopurpurin 4B.**

**Dianthine B,** see **Erythrosin,** bluish.

**Diaphane** for mounting Giemsa preparations (Coulston, F., J. Lab. & Clin. Med., 1940, **26,** 869–873).

**Diaphanol** is according to Lee (p. 598) the trade name for a mixture, formerly obtainable from Leitz, produced by passing chlorine dioxide vapor into ice cold 70% acetic acid. It should be fresh. He advises against attempts to make it and outlines its use in the softening of **Chitin.** Rinse well fixed tissues in 63% alcohol and transfer them to diaphanol until they are softened and bleached. If the diaphanol becomes discolored, repeat. Transfer to 63% alcohol, dehydrate, clear in tetralin (if not available, benzol) and imbed in paraffin. See use of diaphanol in demonstrating **Melanins.**

**Diethylene Dioxide = Dioxan.**

**Differential Leucocyte Count,** statistical study of uniformity in (Klotz, L. F., J. Lab. & Clin. Med., 1939, **25,** 424–434).

**Diffraction Methods** for measuring diameter of red blood cells (Haden, R. L., J. Lab. & Clin. Med., 1937–38, **23,** 508–518).

**Digitonine** reaction of Windaus for *free cholesterol.* This has been adapted to histochemical use by Brunswick and by Leulier and Noel (A., and R., Bull. d'Hist. Appl., 1926, **3,** 316–319). Lison (p. 211) recommends a slight change. Immerse frozen sections of formalin fixed tissue in 0.5% digitonine in 50% alc. for several hrs. Rinse in 50% alc., then in water and mount in Apathy's syrup or glycerin gelatin. With crossed nicols (polarizing microscope) one observes appearance of needles or rosettes of the complex cholesterol-digitonide. To resolve this complex stain with sudan. The esters will color and lose their birefringence while the cholesterol will remain uncolored and retain birefringence.

**Di Nitrosoresorcinol** test for iron, see **Iron.**

**Diotrast,** trade name for an organic iodine preparation recommended by Gross, S. W., Proc. Soc. Exp. Biol. & Med., 1939, **42,** 258–259 for injection into common carotid with later x-ray photographs of the vascular tree.

**Dioxan** is diethylene dioxide. It mixes with water, ethyl alcohol, many clearing agents and paraffin (slightly). McClung (p. 39) recommends its use to replace ordinary agents like xylol. Dioxan fumes are said to be dangerous to laboratory workers so that it should be used under a hood or in a well ventilated room with container covered when not in use (Magruder, S. R., J. Lab. & Clin. Med., 1937–38, **23,** 405–411).

For fixation the following mixtures are recommended (McClung, p. 39): (1) Sat. aq. picric acid, 5 parts; glacial acetic, 1 part; dioxan, 4 parts. (2) Sat. picric acid in dioxan, 4 parts; glacial acetic, 1 part; absolute alcohol, 4 parts. Graupner and Weissberger (von H. and A., Zool. Anz., 1933, **102,** 39–44) suggest: dioxan 80%, methyl alcohol 20%, paraldehyde 2%, and acetic acid 5%. See **Clearing, Pituitary.** See as ingredient of **Lison's** glycogen method; also dioxan imbedding of **Pituitary.**

**Dipeptidase** can be localized in chief cells of stomach. See review of methods (Gersh, I., Physiol. Rev., 1941, **21,** 242–266).

**Di-Phenyl Methane Dyes.** Of these only **auramin** need be referred to.

**Diphtheria Bacilli.** 1. *Neisser's stain* (Stitt, p. 863). *A* = methylene blue, 0.1 gm.; 95% alc., 2 cc.; glacial acetic acid, 5 cc.; aq. dest., 95 cc. *B* = Bismark brown, 0.2 gm.; aq. dest. (boiling) 100 cc. Dissolve and filter. To stain smear pour on *A*, 30–60 sec. Wash. Then *B*, 30 sec. Wash in water, dry and mount. Bacilli brown with dark blue dots at either end. Better results can be secured by adding 1 part of crystal violet (Hoechst) 1 gm.; 95% alc., 10 cc.; aq. dest., 300 cc. to 2 parts of *A* before using. Chrysoidin 1 gm. in hot aq. dest. 300 cc. is more satisfactory counterstain than Bismark brown. Most American brands of crystal violet are satisfactory.

2. *Ponder's stain* (Stitt, p. 863). Toluidin blue (Grubler) 0.02 gm.; aq. dest., 100 cc.; glacial acetic acid, 1 cc.; abs. alc., 2 cc. Add small amount to fixed film on cover glass. Invert and mount on slide. Diphtheria bacilli recognizable by metachromatic granules intensely stained, diphtheroids by their strong color in contrast with ordinary cocci and bacilli the bodies of which are only faintly blue.

3. *Laybourn's modification of Albert's stain* (Stitt, p. 863). *A* = toluidin blue, 0.15 gm.; malachite green, 0.2 gm.; glacial acetic acid, 1 cc.; 95% alc., 2

cc., aq. dest., 100 cc. *B* = iodine crystals, 2 gm.; potassium iodide, 3 gm.; aq. dest., 300 cc. Let both stand 24 hrs. and use filtrate. Apply *A* to heat fixed smears 3–5 min. Wash in water. Apply *B* for 1 min. Wash, blot and dry. Granules of diphtheria bacilli, black; bars, dark green; intermediate parts, light green and all three in sharp contrast.

**Diplosome,** a double centrosome.

**Direct Garnet R,** see **Erie Garnet B.**

**Direct Red 4B,** see **Benzopurpurin 4B.**

**Direct Red, C, R,** or **Y,** see **Congo Red.**

**Direct Sky Blue,** see **Niagara Blue 4B.**

**Direct Steel Blue BB,** see **Dianil Blue 2R.**

**Direct Violet B,** see **Azo Blue.**

**Direct Violet C,** see **Erie Garnet B.**

**Dis-Azo Dyes.** Azo blue, benzopurpurin 4B, Biebrich scarlet, Bismark brown Y and R, brilliant purpurin R, congo red, dianil blue 2R, Erie garnet B, Niagara blue 4B, orseillin, trypan blue, trypan red, sudan III, sudan IV, vital new red, vital red, etc.

**Dissociation,** see **Maceration.**

**Distrene 80** is a polysterene which forms a water clear solution in xylol. It is recommended by Kirkpatrick and Lendrum (J. and A. C., J. Path. and Bact., 1939, **49,** 592–594) as a mounting medium giving good preservation of color in microscopic slides.

**Dominici's Stain,** see **Eosin-Orange G and Toluidin blue.**

**Dopa Oxidase Reaction for Melanoblasts** (Laidlaw, G. F., Anat. Rec., 1932, **53,** 399–407). Dopa is short for 3.4-*d*-oxy-phenylalanin, a substance which when applied in a certain way p cks out the melanoblasts by blackening them. Use frozen sections of fresh material or of tissues fixed 2 to 3 hours but not longer in 5% formalin. Rinse 4 or 5 seconds in aq. dest. and immerse in buffered dopa. (To make dopa stock solution dissolve 0.3 gm. dopa powder—manufactured by Hoffmann-La Roche, Nutley, New Jersey—in 300 cc. cold aq. dest. Keep in refrigerator and discard when solution becomes dark red. To make buffers dissolve 11 gms. disodium hydrogen phosphate ($Na_2HPO_4 + 2H_2O$) in 1000 cc. aq. dest. and 9 gms. potassium dihydrogen phosphate ($KH_2PO_4$) in an equal amount aq. dest. Immediately before use buffer to pH 7.4 by adding 2 cc. potassium phosphate solution, and 6 cc. sodium phosphate solution to 25 cc. dopa solution). The reaction is slow for 3–4 hours at room temperature. If solution becomes sepia brown it is likely to overstain. Observe under microscope. Wash in aq. dest., dehydrate and counterstain if desired with alcoholic crystal violet, clear and mount in balsam. Melanoblasts should be black.

This much used method has been criticized by H. Sharlit et al. (Arch. Dermat. and Syph., 1942, **45,** 103–111) chiefly on the ground that the incubation for 3 hrs. at room temperature may itself increase the amount of melanin present which happened in their experience at 37°C. See also remarks by Blaschko and Jacobson (Bourne, p. 198) on specificity of the reaction. It is given by phenoloxidases but thus far they have not been found in mammalian tissues.

**Double Green,** see **Methyl Green.**

**Double Imbedding.** To facilitate section cutting by making a celloidin block firmer, harden first in chloroform vapor, then in chloroform, transfer to benzol until it becomes transparent and infiltrate with 38°C. paraffin (Lee, p. 104). See **Fleas.**

Another method of double imbedding is that of Péterfi (T., Zeit. f. wiss. mikr., 1921, **38,** 342–345). As employed in this laboratory it is as follows: Make 1% and 3% solutions of celloidin in methyl benzoate which take about a month. Pour some 1% into a dish. Add absolute alcohol containing the tissue which gradually sinks down into the celloidin. Transfer tissue to 3% solution, 48–96 hrs. Drop tissue directly into benzol for a few hrs. Then infiltrate and imbed in 40°C. paraffin about 12–24 hrs.

**Double Scarlet BSF,** see **Biebrich Scarlet,** water soluble.

**Downey's Fluid,** see **Megakaryocytes.**

**Ducts.** These structures lead (L. *ducere*) the products of glands to the site of discharge. They are of considerable variety. Ordinarily they are easily identified by their morphology in hematoxylin and eosin preparations. But special techniques are required for their visualization in whole mounts of some glands.

In the *pancreas* for example the system of small ducts (ductules) can easily be demonstrated by perfusion of the pancreas with pyronin—one of the many methods discovered by R. R. Bensley. Proceed as described under **Perfusion** using a solution made up by adding 10 cc. of 1% aq. pyronin to 1000 cc. 0.85% aq. sodium chloride. When the pancreas has assumed a rose red color the optimum intensity of which must be determined by trials, remove a piece of it, tease out a small lobule and examine under low power mounted in 0.85% aq. sodium chloride. The complicated system of ducts should be sharply delineated by their deep rose

red color in an almost colorless background. If there is any question of their identification examine the original figures of Bensley, R. R., Am. J. Anat., 1911, **12**, 297–388. A double staining of ducts and **Islets of Langerhans** can be obtained by perfusing in the same way with pyronin solution to 1000 cc. of which 6 cc. 1% aq. neutral red has been added. The islets appear yellow red in contrast to the rose red ductules. See, in addition, ducts in whole mounts of **Mammary Glands** and in sections of **Submaxillary Glands** which are of particular interest in detecting the action of salivary gland viruses.

**Ear.** Microscopic examinations of the ear are nearly always made on sections. This is understandable, but it is possible that the study of still living tissues, removed by careful and minute dissections, is a field of considerable promise. The close apposition of epithelial and nervous components to bone necessitates decalcification except in the case of young embryos. The frequent use of celloidin in place of paraffin for imbedding is occasioned by the wide range of diversity in resistance of the organ to the microtome knife, fluid containing lumina being surrounded by hard dense bone. The histological techniques actually in use for the ear are fewer in number and more limited in range than those employed for most other parts of the body. The difficulty experienced in obtaining fresh and normal adult specimens has turned investigation toward human fetuses and the ears of experimental animals.

The fixative usually used is Zenker's fluid with or without acetic acid. Some prefer 10% formalin. In his study of kittens, young rabbits, dogs and rats, Van der Stricht, O., Contrib. to Embryol., Carnegie Inst., 1920, **9**, 109–142 fixed isolated cochleas in 5% aq. trichlorlactic acid, **Bouin's** and **Zenker's** fluids, mordanted for "many weeks" in 70% alcohol + a few drops of iodine solution. After the last 2 fixatives he completed decalcification in 2% nitric acid in 70% alcohol. Before imbedding in paraffin he stained with **Borax Carmine** and he colored the sections with **Iron Hematoxylin, Congo Red** and **Light Green.** Directions will be found in his paper for the demonstration of mitochondria in the sustentacular and hair cells. A differential stain for hair cells is described by MacNaughton, I. P. J., and Peet, E. W., J. Laryng. and Otol., 1940, **55**, 113–114 with a fine colored figure of the results.

The technique of Perlman, H. B., Arch. Otolaryng., 1939, **29**, 287–305, in his study of healing of injuries to the capsule of the labyrinth, is interesting. The use of vital staining with trypan blue to ascertain the nature of cells in the scalae mediae of organs of Corti subjected to trauma is described by Lurie, M. H., Ann. Oto., Rhino., Laryng., 1942, **51**, 712–717. Methods of reconstruction are much employed (Bast, T. H., Arch. Otolaryng., 1932, **16**, 19–38 and others). A simple technique for measuring the length of the basilar membrane is reported by Keen, J. A., J. Anat., 1939–40, **74**, 524–527. See Covell, W. P., Laryngoscope, 1941, **51**, 683–691 on avitaminosis. See **Endolymph.**

**Ectoplasm.** Cytoplasm lying immediately internal to the plasma membrane. It is usually gelled, and, being free from various formed bodies present in the endoplasm, has a clear hyaline appearance.

**Ehrlich-Biondi Stain,** known also as the Ehrlich-Biondi-Heidenhain mixture, is one of the classical stains.

Add 20 cc. sat. aq. acid fuchsin and 50 cc. sat. aq. methyl green to 100 cc. sat. aq. orange G agitating the fluid while doing so. Add 60–100 cc. aq. dest. The diluted mixture should redden slightly if a little acetic acid is added. A drop placed on filter paper should be bluish green at the center and orange at the periphery. If there is an outside red zone too much fuchsin has been used. Stain sections of sublimate fixed tissues 12–24 hrs. Do not wash in water but dehydrate quickly. Clear and mount. This stain gives beautiful results when properly employed but it is fickle. Many helpful suggestions are given in Lee, p. 179.

**Ehrlich's Acid Hematoxylin.** Dissolve 2 gm. hematoxylin in 100 cc. 95% alcohol and add; aq. dest., 100 c.; glycerin, 100 cc.; ammonium (or potassium) alum, 3 gm., glacial acetic acid, 10 cc. Ripen by exposure to air (but not dust) 2 or 3 weeks, or immediately by addition of 0.4 gm. sodium iodate.

**Ehrlich's Triacid** blood stain. This, also, is one of the classic stains, now seldom used. It contains methyl green, orange G and acid fuchsin; but methyl green is a basic dye so that it is not made up of three acid dyes. Ehrlich explained that it is so called "because in it all the three basic groups of the methyl green are combined with acid dye-stuffs" (Lee, p. 167) with which modern chemists do not agree. Air dried smears are fixed by heat (110°C) about 2 min.; stained in triacid (Grübler) 5 min.; washed in aq. dest. until no more color is extracted

and dried with smooth filter paper. Said to color neutrophile granules and leave azur granules unstained.

**Elastic Fibers.** Viewed singly in fresh unstained spreads of **Loose Connective Tissue** these fibers have a faint yellow color, are thinner and more highly refractile than collagenic fibers. Moreover they are optically homogeneous, branch repeatedly to form networks and do not swell when subjected to dilute acetic acid. To demonstrate them in sections a choice can be made from several quite specific stains including **Weigert's Resorcin Fuchsin, Verhoff's Elastic Tissue Stain, Unna's Orcein Method, Krajian's Congo Stain.**

**Elastic Properties,** see **Surface Tension.**

**Electric Tissues** of fishes, methods for are given by Dahlgren (McClung, p. 434).

**Electrical Resistance** and capacity or **Impedence.** By employing alternating currents of varying frequencies figures for apparent resistance and capacity can be obtained. Red cells, yeast cells, ova etc. have been investigated. The technique is not microscopic but the data have an important bearing on structure. In view of the wide variety of cells studied it is interesting, as Danielli remarks (Bourne, p. 42), that a definite pattern should emerge of a cell plasma membrane only $10^{-6}$–$10^{-7}$ cm. in thickness corresponding to a specific resistance of $10^{10}$–$10^{11}$ ohms.

**Electron Microscopy.** Details provided by Dr. W. L. Simpson of The Barnard Free Skin and Cancer Hospital.

1. *Transmitted electron beam type.* The relationship of resolving power (R.P.) to the wave length ($\lambda$) of light employed and to the numerical aperture (N.A.) of a lens system as expressed in the relation R.P. equals $\frac{.5\lambda}{\text{N.A.}}$ proved for many years an apparently insurmountable limitation to the biologist's desire to investigate directly minute structure of cells and tissues. Even when ultraviolet light of 2250Å was employed the limit of resolution was 0.08$\mu$ in a system of N.A. 1.40. With visible light the limit was approximately 0.2$\mu$. On the assumption that the angle of visual acuity is 1 minute of an arc, the greatest magnification that was practical with visible light ranged from 1750 to 2100 times. There is, of course, no limitation to the actual magnification that may be achieved. Increases beyond the limit mentioned, however, do not reveal new structures. As long as this was true there seemed no hope of direct confirmation of the amazing findings made possible by such new methods as x-ray diffraction, ultracentrifugation studies, chemical studies of virus structure, and polarized light methods.

Small wonder is it then that the biologist has grasped with enthusiasm at the possibilities of visualizing ultramicroscopic structure by means of devices that have grown from the fertile field of electron optics. Of most general interest is the electron microscope. With this instrument, using the same equation for resolving power, it should be possible to reach a resolution of at least 0.001$\mu$. Thus, an improvement of at least 200 times over the limit with visible light might be achieved. The practical limit on magnification has been placed at from 70,000 to 100,000 times.

Historically the electron microscope is now fifteen years old. Busch described the first such instrument using magnetic lenses (Busch, H., Archiv. f. Elektroteknik, 1927, **18**, 583–594). Though many improvements were made in design it was not until 10 years later that the instrument reached the point of being of practical use. Chiefly through the work of Ruska and Borries (numerous papers, 1934–1940) the instrument was developed to the state that it is in at present. In this country an excellent instrument, capable of giving high resolution has been developed and commercially marketed by the Radio Corporation of America. The apparatus is described by Zworykin (V. K., Science, 1940, **92**, 51–53). In this instrument electrons emitted from a hot wire filament are accelerated by a potential of 30,000 or more volts. This beam is condensed and passed through the object which is carried on a wire screen supported nitro-cellulose film. The transmitted electron beam is focussed in a greatly enlarged image by means of two magnetic lenses. The image can be seen on a fluorescent screen or photographed on a sensitive plate.

Much work has already been reported on the use of this instrument. Though it is too early to evaluate these new findings, it appears that some of them contribute greatly to our knowledge of the finer structure of viruses (Green, R. H., Anderson, T. F. and Smadel, J. E., J. Med. Research, 1942, **75**, 651–656) and biological fibers, e.g., studies on collagen fibers by Scott and Anderson (G. H. and T. F., Anat. Rec., 1942, **82**, 445) and Schmitt, Hall and Jakus (F. O., C. E., and M. A., J. Cell. and Comp. Physiol., 1942, **20**, 11–33). On the other hand, much of the work appears to be devoted to a simple attempt to see things magnified more than has hitherto been possible. Considerable experience will undoubtedly be required before the

full range of usefulness of such an apparatus can be realized.

2. *Emission electron type.* The earliest description of an electron microscope in this country was of an entirely different type from the new R.C.A. microscopes that give such prodigous magnifications. McMillan and Scott (J. H. and G. H., R.S.I., 1937, **8**, 288–290) published an account of an electron microscope of simple design that used as a source the electron emission of heated sections of tissues. These were accelerated by a potential of 1000 to 2000 volts, focussed by a magnetic lens onto a fluorescent screen. An improved design (Scott, G. H. and Packer, D. M., Anat. Rec., 1939, **74**, 17–29) makes possible magnifications of at least 150 times. By certain modifications the magnification can be increased considerably. It is feasible with this instrument to obtain photographs that record the precise localization of calcium and magnesium salts in tissues. Scott and Packer (*ibid*, 31–45) showed that the calcium and magnesium of skeletal muscle was confined almost entirely to the muscle fibers themselves, and that in contracted fibers a great concentration of magnesium appeared in the contraction nodes.

Tissues to be studied with this technique must be preserved in a manner that permits no redistribution of minerals. The satisfactory method is that of **Altmann-Gersh.**

Electron microscopic technique supplements histospectrography by precisely locating certain elements within tissues and is very useful in conjunction with the technique of microincineration as a means of identifying certain components of the ash seen in sections.

**Electrophoresis** measurements. From data on rate of cell movement in a potential gradient the number of ions per sq. cm. (charge density) on the cell surface can be calculated. For a survey of such measurements see Abramson, H. A. Electrophoretic Phenomena, New York, 1934.

**Eleidin** (G. *elaia*, oil) gives to the stratum lucidum its clear, glassy appearance. It may be a dissociation product of keratohyalin. There has been no great improvement on the specificity of the older methods. Mallory (p. 260) gives the method of Buzzi (1889), first cautioning that fixation must be in formalin, Orth's or Bouin's fluid. Stain frozen sections of 10% formalin fixed tissue in sat. aq. picric acid (approximately 1.2%) 5 min. Rinse in aq. dest. and counterstain for 1 min. in 1% aq. nigrosin. Wash in water and then in 95% alc. (Skip absolute.) Clear in terpineol or origanum oil. Mount in balsam: keratin, bright yellow; eleidin, blue black. Ranvier's **Picro-Carmine** gives a fine red staining of eleidin. See finger **Nails.**

**Elementary Bodies** are the smallest particles of viruses. Those of certain viruses are large enough for direct microscopic examination in suitably stained preparations which usually show also the larger **Inclusion Bodies** if these are present. Various methods designed for **Rickettsia** are usually satisfactory. Many special techniques have been proposed of which 2 follow:

1. Methyl violet or Victoria blue for smears (Gutstein, M., J. Path. & Bact., 1937, **45**, 313–314). Dry smears on perfectly clean slides in air or incubator. If necessary remove excess protein by rinsing in physiological saline solution followed by aq. dest. Fix in methyl alcohol 1 hr. Stain in either of 2 ways: (1) Place slide in Petri dish. Mix equal parts 1% aq. *methyl violet* and 2% aq. $NaHCO_3$. Filter immediately onto the slide, cover dish and incubate at 37°C. 20–30 min. Rinse in aq. dest., dry and mount in cedar oil or liquid paraffin. Elementary bodies light violet. (2) Same except filter onto slide equal parts (a) *Victoria blue* 4R 1 gm., alc. (abs.) 10 cc. and aq. dest. 90 cc. and (b) 0.02% aq. KOH and leave at room temperature over night. Elementary bodies of vaccinia and other viruses dark blue.

2. Methyl blue acid fuchsin for sections (Nicolau, S. and Kopciowska, L., C. r. Acad. d. Sci., 1937, **204**, 1276–1278). Fix in alcoholic Bouin's fluid. Stain 4–5 micron paraffin sections 30–60 min. in: methyl blue (Grübler) 1.5 gm., aq. dest. 65 cc., methyl alcohol 35 cc., glycerin 5 cc., 3% aq. oxalic acid 5 cc. Wash well in aq. dest. and change to absolute alcohol. Stain 20 min. in: acid fuchsin 1.5 gm., aq. dest. 100 cc., 3% aq. oxalic acid 2 cc. Wash directly in absolute alcohol and mount in the usual way. Small particles in cells associated with following viruses: herpes, Borna, Zoster, rabies and pseudo-rabies are stained bright red.

New methods for the collection and purification of elementary bodies permit their direct examination at very high magnifications with the electron microscope (von Borries, E. G., Ruska, E. and H., Klin. Woch., 1938, **17**, 921; Green, R. H., Anderson, T. F., and Smadel, J. E., J. Exp. Med., 1942, **75**, 651–656) and their chemical analysis for vitamin catalysts, copper and enzymes (Hoagland, C. L., Ward, S. M., Smadel, J. E.,

and Rivers, T. M., J. Exp. Med., 1942, **76**, 163–173). See luminescence of elementary bodies (Turevich, E. I. abstracted in Stain Techn., 1941, **16**, 182.

**Ellipsin** is structure protein of cells. Methods for its isolation from liver cells of rabbit and guinea pig by grinding fresh tissue, washing, centrifugation and so on are fully described by Bensley, R. R. and Hoerr, N. L., Anat. Rec., 1934, **60**, 251–266.

**Embedding,** see **Imbedding.**

**Embryological Methods.** In general the techniques which give good results with adult tissues are also satisfactory for embryos; but there are differences as for example in silver impregnations. Moreover greater care is necessary to avoid too sudden changes in the fluids used. Helpful suggestions are given in McClung, pp. 279–286. Application of trichrome staining methods to embryos (Baxter, J. S., J. Anat., 1940–41, **75**, 137–140). See demonstration of **Cartilaginous Skeleton, Ossification** and **Spalteholz** method. Technique for handling chick embryos (Adamstone, F. B., Stain Techn., 1931, **6**, 41–42). Block staining of nervous tissue of embryos with silver (Davenport, H. A., Stain Techn., 1934, **8**, 143–149).

**Enamel** (dental). This can best be studied in ground sections of **Teeth.** 1. *Cuticle.* Wash and brush tooth in tap water. 4% neutral formalin, 24 hrs. Wash tap water, 24 hrs. Mallory's anilin blue (0.5% aq.) 24 hrs. Again wash and brush in tap water. 10% aq. hydrochloric acid, 10 min. As enamel is dissolved delicate opaque white membrane appears. Tease membrane off onto slide coated with egg albumen (**Albumen-Glycerin**). Blot with filter paper. 5% aq. sodium thiosulphate or bicarbonate 10 min. Wash in tap water 10 min. Dehydrate in alcohols, clear in xylol and mount in gum damar (McClung, p. 371).

2. *Rods.* Macerate tooth in 5–10% aq. hydrochloric acid for 24 hrs. Remove a little softened enamel and examine (McClung, p. 372). See Chase, S. W., Anat. Rec., 1927, **36**, 239–258.

3. *Organic Matrix.* Boedeker's method abbreviated from McClung (p. 372). Dehydrate small piece (0.5–1 mm. thick), free from dentin, through alcohols 10 min. each. Methyl alcohol 1–2 hrs. Decalcify in celloidin mixture (parlodion, DuPont) made by dissolving sufficient in methyl alc. C.P. to give thick syrupy solution. To 150 cc. of this add drop by drop constantly stirring nitric acid C.P., 10 cc. + methyl alc. 40 cc. Keep tissue in this mixture in glass dish with air tight cover. Organic matrix appears as brown, spongy material in 10–12 hrs. care being taken to leave the dish stationary. After decalcification is completed, 2–7 days, uncover and permit celloidin to harden. Cut out specimen with narrow margin of celloidin. 70 and 40% alc. 1–2 hrs. each. Aq. sol. alum, 24 hrs. Running water, 6–12 hrs. Ascending alcohols to 95% 1–2 hrs. each. Anilin oil, 6–12 hrs. (becomes brown and transparent). Equal parts anilin oil and chloroform, 6–12 hrs. Imbed in paraffin not over 52°C. Mount 3–10$\mu$ sections, dry and treat with xylol 3 min. Dissolve celloidin in ether-alcohol. Abs. alc. 1 min. Descending alcohols to water. Stain in **Iron Hematoxylin.**

4. *Cape-Kitchin celloidin decalcification method.* Cut DuPont's parlodion into small cubes and dissolve in acetone free methyl alcohol making thick solution. To 200 cc. add 90 cc. methyl alcohol constantly stirring and 9 cc. nitric acid, sp. gr. 1.42. Follow decalcification of enamel in this mixture between crossed nicols of polarizing microscope with 24 mm. objective. Double refraction disappears with decalcification (Bödecker, C. F., J. Dent. Res., 1937, **16**, 143–150).

5. *Permeability.* When the apex of a tooth is immersed in strong alcoholic solution of fuchsin + NaCl the enamel becomes stained (v. Beust, T., Dental Cosmos, 1912, **54**, 659). Another way is to test for penetration of lead, boron and other easily recognizable chemicals (Howe, P. R., Dental Cosmos, 1926, **68**, 1021–1033). After intraperitoneal injections of trypan blue blue coloration can be observed in developing enamel only (not adult) as well as in dentin of dogs (Gies, W. J., J. Nat. Dent. Assoc., 1918, **5**, 529–531). Marshall (J. S., J. Dent. Res., 1921, **3**, 241–255) employed Naphthamine brilliant blue similarly as a vital stain. See **Dentin,** vital staining.

**Endamoeba.** This genus includes *E. histolytica*, the cause of amebic dysentery and *E. coli* and *E. gingivalis*, two apparently harmless commensals. The technique is essentially the same for all three. In searching for E. histolytica or E. coli take a small amount of fresh feces, mix with physiological saline solution and examine directly. Recognize amebae by large size and movements if slide is kept warm. E. histolytica frequently contains erythrocytes. Mallory (p. 296) advises mixture with **Gram's Iodine** solution to demonstrate glycogen if present, or mixing with drop 1–2% formalin, then treatment with drop 2% acetic acid and coloration with

1 drop 1% aq. neutral red. E. gingivalis is to be found in decayed teeth. Only E. histolytica extensively invades tissues.

1. To make permanent smear preparations (Mallory, p. 296) fix thin smear while moist in 95% alcohol, 1 part, and sat. aq. corrosive sublimate, 2 parts, for 15 min. Wash for few sec. in water and cover with 1% alcoholic iodine for 3 min. Wash in aq. dest. until iodine color is extracted. Wash again and stain with **Phosphotungstic Acid Hematoxylin,** 30 min. Wash in water, dehydrate in 95 and abs. alcohol, clear in xylol and mount in balsam. Nuclei and ectosarc, deep blue; cytoplasm, bluish.

2. To stain differentially in sections (Mallory, p. 297). Fix in 95% or abs. alc., and make paraffin or celloidin sections. Stain in 0.25% aq. thionin 3–5 min. Differentiate in 2% aq. oxalic acid, ½–1 min. After washing in water, dehydrate in 95% and abs. alc. Clear in xylol and mount in balsam, except for celloidin sections which require clearing in terpineol, or origanum oil, after 95% alc. Nuclei of amebae brownish red, those of all other cells, blue. See **Amebae.**

**Endolymph.** To demonstrate its circulation employ method used by Guild, S. R., Am. J. Anat., 1927, **39**, 57–81. Introduce solution of potassium ferrocyanide and iron ammonium citrate into cochlear ducts of living guinea pigs under anesthesia. Kill at intervals up to 48 hrs. Excise tissue and fix in acid fluid which precipitates **Prussian Blue** wherever the solution has circulated.

**Endospore** stain for bacteria in blood smears. Smear, air dry and fix by flaming 3 times. 5% aq. malachite green 5 min., wash in tap water 10–20 sec. 0.5% aq. safranin, 10 sec., wash quickly, dry and examine (Bruner, D. W. and Edwards, P. R., J. Lab. & Clin. Med., 1939, **25**, 543–544).

**Enrichment** techniques, see **Concentration.**

**Enterochromaffin Cells,** influence of pilocarpin on (Hamperl, H., Ztschr. f. mikr.-anat. Forsch., 1925, **2**, 506–535). See **Small Intestine.**

**Enzymes.** Their name is legion. At present only a few can be localized histochemically within or near their cells of origin. There is no better example of advantageous association between histological and biochemical methods. At present four principal kinds of technique are employed for localization: (1) By spectrographic identification in the tissues—especially the **Cytochrome Oxidases,** (2) By close comparison of enzymatic properties with cellular composition of the tissues—**Amylase, Pepsin, Peptidase, Esterase, Protease, Cholinesterase, Lipase, Urease, Carbonic Anhydrase,** etc., (3) By separation of nuclei from cytoplasms by differential centrifugation and by estimation of enzyme in each—**Arginase,** (4) By the development of characteristic products within the cells or tissues—**Cytochrome Oxidase, Oxidase, Phenolase, Peroxidase, Phosphatase, Dopa Oxidase.** See also **Nuclease, Cathepsin, Lysozyme** and **Adenosinase.** The terms lyo- and desmo-enzymes are used to indicate respectively the enzymes which can and cannot be separated from cell proteins.

Whether dyes are of any service as indicators of the presence of enzymes remains to be determined. However Robertson, T. B., J. Biol. Chem., 1906, **2**, 317–383, found that a little safranin added to a solution of trypsin forms a colored ppt. and Holzberg, H. L., J. Biol. Chem., 1913, **14**, 335–339 observed that the ppt. exhibits proteolytic activity, and Marston, H. R., Biochem. J., 1923, **17**, 851–859, discovered that azure dyes, including neutral red, likewise precipitate pepsin, trypsin, crepsin and papain. The linkage of enzyme to dye is, he thinks, through the basic nitrogen of the heterocyclic ring of the latter. In view of these observations, and the coloration of mitochondria with janus green, Marston suggests that the mitochondria contain proteolytic enzymes. Methods for the enzymatic analysis of purified elementary bodies of vaccinia are described by Hoagland, C. L., Ward, S. M., Smadel, J. E., and Rivers, T. M., J. Exper. Med., 1942, **76**, 163–173. Two very helpful reviews are recommended: Gersch, I., Physiol. Rev., 1941, **21**, 242–266, and Blaschko, H. and Jacobson, W. (Bourne, pp. 189–224).

**Eosinophile Leucocyte** (acidophilic or coarsely granular leucocyte). Can easily be examined while still living in mounts of fresh blood. The dark field is useful. Most frequently studied in **Blood Smears,** which see. Mitochondria are readily stainable with **Janus Green.** For occasional presence of basophile granules and pigment see Downey, H., Folia Haemat., 1915, **19**, 148–206. Techniques for rapid experimental increase of eosinophiles in circulating blood are described by Banerji, N., Am. J. Med. Sci., 1933, **186**, 689–693; Chillingworth, F. P., Healy, J. C. and Haskins, F. E., J. Lab. and Clin. Med., 1933–34, **19**, 486–494; Hajos, K., Németh, I., and Enyedy, Z., Zeit. f. d. ges. Exper. Med., 1926, **48**, 590–592.

**Eosins.** There are several fluorescein dyes

and guidance may be needed in the choice of the one best suited for a particular purpose. Conn, H. J. and Holmes, W. C., Stain Tech., 1926, **1**, 87–95; 1928, **3**, 94–104 have made a study of color, acidity and chemical structure and Conn (p. 145) gives further data. Their color increases in depth in this order: eosin Y, ethyl eosin, eosin B, erythrosin B, phloxine and rose bengal. This increase in color is proportional to increase in number of hologen atoms. Their acidity increases in a different order: rose bengal, phloxine, erythrosin, eosin Y and eosin B. (1) When the eosin is to follow in alcoholic solution a basic dye always in aqueous solution (cf. hematoxylin) the more acid and lighter colors are recommended (eosin Y, ethyl eosin and eosin B. (2) When it is to precede in aq. solution a basic dye (cf. methylene blue) also in aq. solution, use phloxin or erythrosin (see **phloxine-methylene blue**).

**Eosin B** or bluish (CI, 771)—eosin BN, BW, or DHV, eosin scarlet, eosin scarlet B, imperial red, nopalin G, saffrosin, scarlet J, JJ, V—Dibrom derivative of dinitro-fluorescein. Chemistry of (Holmes, W. C., Melin, C. G. and Paterson, H. R., Stain Techn., 1932, **7**, 121–127).

**Eosin 10B,** see **Phloxine B.**

**Eosin BN, BW,** or **DHV,** see **Eosin B** or bluish.

**Eosin J,** see **Erythrosin,** bluish.

**Eosin-Methyl Blue,** see **Mann's.**

**Eosin-Methylene Blue** has been employed in many combinations for years. But when the acid dye is applied first, phloxine is preferred to eosin. See therefore **Phloxine Methylene Blue.**

**Eosin-Orange G—Toluidine Blue** for bone marrow, spleen and connective tissue (Dominici, M. C. rend. Soc. biol., 1902, **54**, 221–223). Stain eosin-orange G (eosin B. A. of Hollborn or eosin yellowish of American manufacturers 0.5 gm.; aq. dest., 100 cc.; orange G. 0.5 gm.) 7 min. Rinse quickly in aq. dest. Counterstain in 0.5% aq. toluidin blue 20–30 sec. Rinse again aq. dest. Differentiate in 95% alc., dehydrate in abs., clear in xylol and mount in balsam. Instead of eosin, 0.5% aq. acid fuchsin gives a little sharper contrast. In place of toluidin blue 0.1% Azur A can be employed to advantage. Phloxine-orange G can be tried as a substitute for eosin-orange G. (phloxine 0.12 gm., aq. dest. 100 cc., orange G, 0.3 gm.). The crucial point is the differentiation in 95% alc. This should be quickly checked under the microscope until the time has been determined.

**Eosin Scarlet,** see **Eosin B** or bluish.

**Eosin Scarlet B,** see **Eosin B** or bluish.

**Eosin Y** or yellowish (CI, 768). Tetrabrom fluorescein with some mono- and di-brom compounds. This is the usual kind of eosin employed.

**Epidermis.** This can be studied in situ with the dermis, see **Skin,** or it can be examined in 3 ways apart from the dermis.

1. *Isolated pieces.* Examination of scrapings of the epidermal surface is of limited usefulness in special cases. To cut away a few of the deeper cells, separate them by teasing and to study them in the still living state with or without supravital stains is not particularly helpful. But their microdissection is capable of giving important data on cellular consistency and connections (Chambers, R. and deRenyi, G., Am. J. Anat., 1925, **35**, 385–402 and Thanhoffer, L., Zeit. f. Anat. u. Entw., 1933, **100**, 559–562). Their cultivation is possible, see **Tissue Culture.**

2. *Whole mounts* for microscopic study (Cowdry's Histology, p. 530). Place excised fresh skin in 1% acetic acid in ice box for 12–36 hrs. depending upon size, age and region. Wash in tap water, 5 min. Pin skin down with epidermis up and cover with water. Strip off epidermis as a compete sheet. Wash in aq. dest., 5 min. Stain in Harris' hematoxylin, 20 min. Wash in aq. dest., 1 min. Differentiate in 50 cc. 70% alcohol plus 3 drops hydrochloric acid until epidermis becomes light pink color. Treat with 50 cc. aq. dest. plus 6 drops ammonia until it becomes blue. Wash in aq. dest. 5 min. several changes. Dehydrate in 50, 70, 95 and 2 changes of absolute alcohol, 10 min. each. Clear in 2 changes xylol, 1 hr. each and mount in balsam inner side up.

If the skin is hairy, before excising it, remove hair with scissors and electric razor or depilatory solution. Hair follicles and sebaceous glands, unless particularly large, generally remain attached to the epidermis, but the coiled bodies of the sweat glands are too deeply situated to come off with it. Consequently only their straight ducts are to be seen. Before dehydration, in the above technique the sebaceous glands can be sharply counterstained with **Sudan III.**

Such whole mounts of epidermal sheets are of value insofar that their study gives a concept of the morphology of the epidermal covering of the body which can be obtained in no other way. For the counting of mitoses they are far better than sections and have been extensively employed for this purpose by Dr. Cooper and her associates in The

Barnard Free Skin and Cancer Hospital. See her latest paper (Cooper, Z. K. and Reller, H. C., J. Nat. Cancer Inst., 1942, **2**, 335-344). Since the mucous membrane covering the nasal septum can be similarly prepared as a whole mount it is likely that the method may be of service in the study of other sheets of epithelial cells.

3. *Sheets of epidermis* for chemical analysis. Until very recently the handicap experienced in chemical analysis of the skin has been the difficulty of separating epidermis and dermis by themselves for analysis. All data on the epidermis are of doubtful value because variable amounts of dermis have been included. The method of obtaining pure epidermis by dilute acetic acid separation is unsatisfactory for numerous reasons. Baumberger, J. P., Suntzeff, V. and Cowdry, E. V., J. Nat. Cancer Inst., 1942, **2**, 413-424 have discovered that dilute alkali will serve as well as dilute acetic but this also is objectionable from the chemical point of view. They therefore advance a heat method. Place excised skin with dermis down on warm plate such as is used for mounting paraffin sections. Apply temperature of 50°C. for 2 min. which loosens the epidermis so that it can be easily pushed off with a blunt instrument. Separation is more difficult when temperature is over 51°C. Epidermises removed in this way for a time continue to consume oxygen and are very suitable for chemical analysis. They have been used for epidermal iron and ascorbic acid by Carruthers, C. and Suntzeff, V., J. Nat. Cancer Inst., 1942, **3**, 217-220, and for total lipid-protein nitrogen ration by Wicks, L. F. and Suntzeff, V., **3**, 221-226.

**Epinephrin** (adrenin, adrenalin), hormone of adrenal medulla.

**Erie Fast Yellow WB,** see **Titan Yellow.**

**Erie Garnet B** (CI, 375).— amanil garnet H, Buffalo garnet R, Congo corinth G or GW, corinth brown G, cotton corinth G, diamine Bordeaux CGN, direct garnet R, direct violet C — an acid disazo dye used for staining frozen sections (Geschickter, C. F., Stain Techn., 1930, **5**, 81-86).

**Erhlicki's Solution.** Potassium bichromate, 2.5 gm.; copper sulphate, 1 gm.; aq. dest., 100 cc. Used for hardening nervous tissues.

**Erythroblasts,** see **Erythrocytes, Developmental Series.**

**Erythrocyte Counts** do not fall in the scope of this book. It is sufficient to state that they are going out of fashion because of the large experimental error involved and since it is so easy to detect variations in shape, size and maturity of erythrocytes in smears and to measure hemoglobin content of blood by hemoglobinometers. See **Reticulocytes.**

**Erythrocytes.** For chemical and physical studies erythrocytes are particularly adapted, because they can be collected in enormous numbers free from other kinds of cells and from intercellular substances. In order to determine marked differences in size and shape and hemoglobin content examination of fresh blood with direct illumination, or in the dark field, is probably the best procedure. An interesting photographic method for the stereoscopic visualization of the shape of erythrocytes has been described and illustrated by Haden, R. L., J. Lab. & Clin. Med., 1936-37, **22**, 1262-1263. For more accurate techniques see Wintrobe, M. M., Clinical Hematology, Philadelphia: Lea & Febiger, 1942, 792 pp. A new *anisocytosis* index is proposed by van den Berghe, L., and Weinberger, E., Am. J. Med. Sci., 1940, **199**, 478-481. The *refractile body* of Isaacs (R., Anat. Rec., 1925, **29**, 299-313) can also be well studied in fresh blood. See **Flagella.**

Smears, colored by Giemsa or Wright's stain, are satisfactory for *Howell-Jolly bodies*, *Cabot rings*, *basophilic stippling* and *polychromatophilia*. For resistance to hemolysis in hypotonic sodium chloride solutions, see Daland, G. A., and Worthley, K., J. Lab. & Clin. Med., 1934-35, **20**, 1122-1136. A lysolecithin fragility test is described by Singer, K., Am. J. Med. Sci., 1940, **199**, 466-477. For *microfragility tests* see Kato, K., J. Lab. & Clin. Med., 1940, **26**, 703-713 and for *basophilic erythrocytes* of the newborn see McCord, C. P., and Bradley, W. R., Am. J. Clin. Path., 1939, Tech. Suppl., **2**, 329-338. A thorough investigation of erythrocytes in fetus and newborn has been made by Wintrobe, M. M. and Schumacker, H. B., Jr., Am. J. Anat., 1936, **58**, 313-328. A simple method for determination of specific gravity of erythrocytes is described by Reznikoff, P., J. Exper. Med., 1923, **38**, 441-444. After hemolysis the stroma remains and can be studied microscopically or chemically. Lipid analyses are particularly significant (Erickson, B. N., et al., J. Biol. Chem., 1937-38, **122**, 515-528).

Experiments have been made with radioactive iron as a means of tagging red blood cells (Cruz, W. O., Hahn, R. F., Bale, W. F. and Balfour, W. M., Am. J. Med. Sci., 1941, **202**, 157-162) which open up a new field for study of age changes because the cells are thereby dated. Special methods are given under

**Hemoglobin, Flagella and Reticulocytes.**

**Erythrocytes, Developmental Series.** The technique employed apparently makes a great deal of difference in the conclusions reached. See Cowdry's Histology, p. 99.

1. Maximow and Bloom employing mainly permanent preparations list:

*Hemocytoblasts:* "... large (up to 15$\mu$) ameboid, non-granular basophil cells of lymphoid nature." Occur extravascularly.

*Basophil erythroblasts:* The youngest erythroblasts, characterized by the intense basophilia of their cytoplasm. Also called megaloblasts, but "this term is misleading because it was first used for the erythroblasts of pernicious anemia which are cells of quite different nature."

*Polychromatic erythroblasts:* So-called because after "fixation and staining with the Romanowsky mixture, especially in dry smears, the protoplasm has a mixed color varying from purplish-blue to lilac or gray." This is due to the presence of two substances, a basophile material and hemoglobin.

*Orthochromatic erythroblasts* or normoblasts: These are smaller "and only slightly larger than the mature, non-nucleated erythrocytes." Since the basophile substance diminishes and the hemoglobin increases, the protoplasm becomes acidophilic "and stains a bright pink with the Romanowsky mixture." They continue to divide mitotically for an unknown number of generations until the nucleus disappears.

2. Sabin and associates relying chiefly on supravital stains list:

*Endothelial cells:* Occur in special "erythrogenic capillaries."

*Megaloblasts:* "... a daughter endothelial cell which starts to synthesize hemoglobin." "The megaloblast has maximum basophilia, a moderate number of rod-shaped mitochondria, a trace of hemoglobin, and a nucleus with a minimum of chromatin and conspicuous nucleoli."

*Early erythroblasts:* "The young erythroblast represents a growth phase, with less rapid division, for the cell is much larger than the megaloblast; it contains the maximum number of mitochondria. The amount of hemoglobin is still small, but sufficient to give a polychromatophilia, predominately basophilic in methylene blue-azur. The nucleus has a marked increase in chromatin."

*Late erythroblasts:* This cell "is intermediate in size between the early erythroblast and the definitive red cell. The nucleus has lost the nucleoli but still has massive chromatin. . . . The increase in hemoglobin is marked and in fixed films the cytoplasm is more acidophilic."

*Normoblasts:* "The stage of the normoblast is defined as a nucleated red cell after its last cell division. It has a small pyknotic nucleus ready for extrusion or fragmentation."

**Erythrosin B,** see **Erythrosin,** bluish.

**Erythrosin BB** or **B extra,** see **Phloxine.**

**Erythrosin,** bluish (CI, 773)—dianthine B, eosin B, erythrosin B, iodeosin B, pyrosin B—Fluorescein with 2 iodine atoms. See **Eosins.**

**Esterase,** see method under **Lipase.**

**Ethyl Eosin** (CI, 770). The ethyl ester of eosin Y. Sold often as alcohol soluble eosin. See **Eosins.**

**Ethyl Green** (CI, 685). This is, like methyl green, prepared from crystal violet but differs from it insofar that an ethyl group is added instead of a methyl one. For most purposes it is a satisfactory substitute for methyl green.

**Ethyl Purple 6B,** see **Ethyl Violet.**

**Ethyl Violet** (CI, 682)—ethyl purple 6B—It is hexaethyl pararosanilin, a basic dye employed by Bowie, D. J., Anat. Rec., 1924, **29,** 57 to make a neutral stain with biebrich scarlet for staining islets of Langerhans of fish. Kernohan, J. W., Am. J. Clin. Path., 1931, **1,** 399–403 has used in Heidenhain's modification of Mallory's ethyl-violet orange G after formalin fixation.

**Ethyl Violet-Biebrich Scarlet,** see **Bowie's** stain for pepsinogen.

**Ethylene Glycol Mono-Ethyl Ether = Cellosolve.**

**Eunematoda,** see **Parasites.**

**Euperal** is, according to Lee (p. 227), a mixture of camsal, eucalyptol, paraldehyde and sandrac, $n$ = 1.483 of two sorts colorless and green. Since the green one contains a copper salt it strengthens hematoxylin stains.

**Evans Blue** (T. 1824 Eastman Kodak Co.). Used clinically in man for estimation of blood volume. Vital staining of malignant tumors in man (Brunschwig, A., Schmitz, R. L., and Clarke, T. H., Arch. Path., 1940, **30,** 902–910). It is not taken in by red cells and hence is valuable for the determination of plasma volume (Gregersen, M. I., and Schiro, H., Am. J. Physiol., 1938, **121,** 284–292. See **Blood Cell Volume.**

**Excelsior Brown,** see **Bismark Brown Y.**

**Excretion** contrasted with secretion (Cowdry's Histology, p. 259).

**Extracellular** fluid or phase, see **Chloride.**

**Eyes.** Techniques easily used for other parts of the body require special care in the case of the eye. When sections

through the entire eye are required it is important to see that the fixative chosen penetrates properly and that the normal shape of the organ is retained. Fixation by vascular injection may be helpful but it is not sufficient because so much of the eye is avascular. After removal of the eye from the orbit, whether previously injected or not, and after the dissecting away of unwanted muscular and other tissues, it should be immersed in the fixative. This will harden the outer coats somewhat. After a few minutes small amounts of the fixative should be injected by a hypodermic syringe into both chambers choosing locations not in the plane of the proposed sections and providing opportunity for fluid also to leave. Then, with a sharp razor blade, a deep cut should be made to permit free entrance of the fixative. After several hours, more of the tissue on either side of the plane should be cut away. Imbedding in celloidin by the rapid method is preferable to paraffin since it affords much needed support to the less dense parts. Orientation for sectioning is also easier in celloidin because one can see through it fairly well.

If, on the other hand, preparations are needed of small parts of the eye these parts should be carefully dissected out and the paraffin technique employed. Much time will be saved by following the excellent suggestions made by S. L. Polyak, The Retina. Univ. of Chicago Press, 1941, 607 pp. and by G. L. Walls (Stain Techn., 1938, **13**, 69–76). For the investigation of permeability, oxidation-reduction potential, enzyme systems, and such properties, see Friedenwald, J. S. and Stiehler, R. D., Arch. Ophth., 1938, **20**, 761–786. Useful data are to be found in Kurze's Handbuch der Ophthalmologie (Schieck and Brückner, Berlin: Julius Springer, 1930, **1**, 882 pp.) The **Anterior Chamber** is a favourite site for tissue transplantation.

Frozen sections of bird's eyes. (Oakley, C. L., J. Path. & Bact., 1937, **44**, 365–368). Fix in 10% formol saline 4 days, in Müller's fluid, 6 weeks in incubator, or, in case speed is necessary, in Perdrau's fluid 4 days. Incise large eyes to aid penetration. Wash in running water at least 24 hrs. because formalin and bichromate should be completely removed. Cut eye in half being careful not to disturb various structures. 12.5% gelatin + 1% phenol over night, 25% 24 hrs. at 37°C. Employ at least 25 cc. for each half eye. Mount with cut surface down in dish containing 25% melted gelatin. Set overnight in running water or in icebox (not refrigerator). Cut out block, trim away excess gelatin. Harden in large amount 10% formalin, 2–3 days, store in 4% formalin. Before freezing soak 15 min. in tap water. Freeze slowly, over-freeze and then stain usual methods but carefully avoid strong alcohols. They will stand 70% and 1% HCl provided washing in water has been thorough. Use glycerin jelly for mounting.

**Fahrenheit Temperature** to Centigrade. Use the following relation:

$$\tfrac{5}{9}(F° - 32) = C°$$

$$302\ °F \pm \tfrac{5}{9}(302 - 32) = \tfrac{5}{9}(270) = 150°\ C.$$

$$.5°F \pm \tfrac{5}{9}(5 - 32) = \tfrac{5}{9}(-27) = -15°C.$$

$$-13°F \pm \tfrac{5}{9}(-13 - 32) = \tfrac{5}{9}(-45) = -25°C.$$

**Fallopian Tubes** (oviducts, uterine tubes). References to many techniques will be found in C. G. Hartman's chapter in Allen, Danforth and Doisy's Sex and Internal Secretions. Baltimore: Williams and Wilkins, 1939, 1346 pp.

**Farrant's Medium.** Gum arabic, 30 gm.; glycerin, 30 cc.; arsenous oxide (arsenic trioxide), 0.1 gm.; aq. dest., 30 cc. (McClung, p. 617).

**Fast Acid Blue R** (CI, 760). An acid xanthene dye. Conn (p. 143) says that it is almost the same as violamine 3B which contains small amount of a red dye. See Romell, L. G., Stain Techn., 1934, **9**, 141–145 under **Soil**, bacteria.

**Fast Acid Green N**, see **Light Green SF** yellowish.

**Fast Blue B, OB, R,** etc., see **Indulin,** water soluble.

**Fast Blue 3R**, see **Naphthol Blue R.**

**Fast Fuchsin G**, see **Chromotrope 2R.**

**Fast Green FCF.** Commission Certified. Closely related to **Light Green SF** yellowish and recommended as a substitute because it fades less.

**Fast Oil Orange II**, see **Oil Red O.**

**Fast Red**, see **Amaranth.**

**Fast Red B, BN** or **P**, see **Bordeaux Red.**

**Fast Violet**, see **Gallocyanin.**

**Fast Yellow** (CI, 16)—acid yellow, fast yellow FY, G, S, BG, etc.—An acid mono-azo dye. Employed by several investigators, see use by Wallart, J. and Houette, C., Bull. d'Hist. Appl., 1934, **11**, 404–407 in rapid trichrome hematoxylin, acid fuchsin fast yellow method. They used "Jaune solide G or GG (Ciba).

**Fasting.** Structural changes in human digestive tract (Cowdry's Histology, p. 305).

**Fat Blue B**, see **Victoria Blue B.**

**Fat Blue 4R**, see **Victoria Blue 4R.**

**Fat Ponceau**, see **Oil Red O.**

**Fat Ponceau**, see **Sudan IV.**

**Fat Ponceau G**, see **Sudan III.**

**Fat Ponceau R** or **LB**, see **Sudan IV.**

**Fats**, see **Lipids.**

**Fatty Acids,** see **Lipids,** examination with polarized light, also lack of specificity of blue color with **Nile Blue Sulphate.** A review of the method of tagging fatty acids with radioactive isotopes is given by Bloor (W. R., Physiol. Rev., 1939, **19,** 557–577).

**Feathers,** see **Ceresin** imbedding.

**Feces.** 1. To demonstrate ova of parasites (Mallory, p. 301). If they cannot be seen when a small bit of feces is mixed with water on a slide attempt to concentrate them. To a small amount of feces add sufficient sugar solution (common granulated sugar, 500 gm., water, 360 cc., phenol, 1%) to almost fill tube. Cover and gently mix contents. Centrifuge at 1000 r.p.m. 5–6 min. Remove material from surface in wire loop and examine microscopically for ova. Another method is to use hypertonic salt solution in proportion to feces of not more than 20:1 in the same way, removing large particles as may be necessary before centrifuging.

2. To find segments and whole adult worms. Wash feces in small amount water through medium mesh screen, collect and examine at low magnification. For identification consult a text book of parasitology.

**Ferments,** see **Enzymes.**

**Ferric Chloride-Osmic Acid** for demonstration of Golgi apparatus (Owens, H. B. and Bensley, R. R., Am. J. Anat., 1929, **44,** 79–100). Fix and impregnate each piece of tissue 7–10 days at 37°C. in ferric chloride, 0.05 gm.; 2% osmic acid, 10 cc.

**Ferrihemate,** see **Hematin.**

**Fettblau** -braun, -grün, -orange, -rot and -schwartz. These are lipid stains of Hollborn. For use of hydrotropes (Hadjioloff, A., Bull. d'Hist. Appl., 1938, **15,** 37–41).

**Feulgen Reaction,** see **Thymonucleic Acid.**

**Fibers.** Many are recognized. See **Nerve, Collagenic, Reticular, Elastic, Neuroglia.** Muscle fibers are given under **Muscle.**

**Fibrils.** These are really small fibers many of which are intracellular. See **Neurofibrils, Myofibrils, Epidermal Fibrils, Fibroglia, Myoglia.**

**Fibrin.** Usually easily identifiable in **Hematoxylin** and **Eosin** preparations. Weigert's (1887) standard differential stain for fibrin may be used as advised by Mallory (p. 193). Paraffin sections of material fixed in abs. alcohol, **Carnoy** or **Alcohol-Formalin** can be employed. If the fixative contains chrome salts **(Zenker, Helly)** treat first with 0.25% aq. potassium permanganate, 10 min., then 5% aq. oxalic acid, 20 min. and wash in aq. dest. Stain nuclei with **Lithium Carmine.** Mix 3 cc. of A: abs. alc., 33 cc.; anilin oil, 9 cc. saturated with methyl violet (crystal violet) with 27 cc. of B: sat. aq. methyl violet. Stain 5–10 min. Drain and blot. Treat sections with **Gram's Iodine,** 5–10 min. Drain and blot. Differentiate in equal parts anilin and xylol drop by drop until purple ceases to be removed. Blot and remove anilin with xylol. Mount in balsam. Fibrin blue-black, nuclei red.

**Fibroblasts.** There is no specific stain for fibroblasts. In fresh spreads of **Loose Connective Tissue** they are fairly conspicuous elements identifiable by their large usually slightly kidney shaped nuclei (possessed generally of a single nucleolus) and tapering cytoplasmic processes devoid of specific granulations. In sections less cytoplasm is seen and it may be impossible in some cases to identify the nuclei with assurance. Recognition is mainly by position and the exclusion of other possibilities. View the beautiful colored plates of Evans, H. M. and Scott, K. T., Contrib. to Embryol., Carnegie Inst., 1922, **47,** 1–55 for a comprehensive picture of the responses of fibroblasts to vital stains. Pure strains of fibroblasts can easily be cultured, their behavior watched and their nutritional and other requirements investigated. See **Tissue Culture.**

**Fibroglia Fibrils.** Mallory's **Phosphotungstic Acid Hematoxylin** stain for.

**Fibrous Connective Tissue.** Since this is much denser than **Loose Connective Tissue** the method of making spreads is not feasible. It can best be examined in sections of **Zenker** fixed material colored by **Mallory's Connective Tissue Stain** supplemented by specific stains for **Elastic Fibers.**

**Filament-Nonfilament Count.** Neutrophilic leucocytes are divided into two classes: *filament,* in which nuclear segments are connected by delicate strands consisting apparently of nuclear membrane only and *nonfilament* in which there are no filaments the strand being so coarse that it may be resolved into nuclear membrane plus nuclear contents. The former are mature and the latter are less differentiated cells. According to Pepper, O. H. and Farley, D. L., Practical Hematological Diagnosis, Philadelphia, Saunders, 1933, 562 pp., 8–16% of neutrophiles are normally nonfilament cells. A shift to the right is a decrease in this percentage. The count is easier to make than the Arnett or Schilling count and is probably of equal value. See also **Nonfilament-Filament Ratio.**

**Filterable Viruses,** see **Victoria Blue 4B** and **Virus.**

**Fischler's** modification of Benda's stain for *fatty acids* and *soaps* (Fischler, F., Zentralbl f. Allg. Path. u. path. Anat., 1904, 15, 913-917) has been severely criticized by Lison (p. 203) who concludes that it is of no microchemical value.

Mallory (p. 120) has, however, given a somewhat different description of the technique. He explains that since the Na and K fatty acid salts (*soaps*) are soluble in formalin, it is necessary to change them into insoluble Ca soaps by saturating the 10% formalin fixative with calcium salicylate. Comparison of stained sections of such material with others fixed simply in formalin shows the presence and absence of the fatty acid salts (soaps). Calcium soaps can be distinguished from *fatty acids* because they resist solution in a mixture of equal parts abs. alc. and ether or in hydrochloric acid whereas the fatty acids are soluble in this mixture and calcium in hydrochloric acid. The method, as detailed by Mallory, is: Mordant frozen sections of 10% formalin fixed material in sat. aq. copper acetate (12.5%), 2-24 hrs. at room temperature. Wash in aq. dest. Stain 20 min. or more in Weigert's hematoxylin made up by mixing 1 gm. hematoxylin dissolved in 10 cc. abs. alc. with 1 cc. sat. aq. lithium carbonate (about 1.25%) plus 90 cc. aq. dest. several days before use. Differentiate in Weigert's borax-potassium ferricyanide, (2.5 gms. ferricyanide and 2 gm. borax plus 100 cc. aq. dest.) much diluted until red blood cells become decolorized. Wash thoroughly in aq. dest. Mount in glycerin jelly or glycerin. Fatty acids deep blue black. Fe, Ca and hemoglobin may also be stained. To stain *neutral fats* in addition stain with scarlet red after washing out Weigert's fluid, rinse in 70% alc. and in water and mount in glycerin.

**Fixation** by immersion is usually the first step in making permanent preparations. Compared with the direct microscopic examination of still living cells removed from the body and placed in approximately isotonic media, it has both advantages and disadvantages. Among the first is the fact that the normal form relations of the tissue components are more faithfully preserved in large pieces by fixation; because it is not necessary to separate the tissue by teasing, or in some other way, into sufficiently small or thin pieces for microscopic study. Moreover, by fixation, the cells are suddenly and uniformly killed, so that the changes resulting from unfavorable fluid environment outside the body, leading slowly or quickly to injury and death, are not encountered. The chief objection to fixation is that the structure is very definitely modified thereby and care must be exercised in reaching conclusions as to living tissues from the study of fixed ones. It is important to restrict these structural changes to those inseparable from the action of the fixative itself, and of the subsequent technique under the most favorable conditions.

Reduce to a minimum the time in which these complicating alterations can occur by *prompt* fixation. Remove the tissue from an animal under general anesthesia, or immediately after it has been killed, by a method unlikely to injure the tissues. In the case of human tissues removed at operation one should be on the look out for complicating factors. If the tissue is collected at autopsy the autopsy should be made at the earliest possible moment after death. See **Postmortem Changes.** If delay is unavoidable, keep the body, or the tissue, in an ice box to reduce the speed of chemical change. In case an excised tissue cannot be immediately fixed, place it in a covered glass container with some cotton moistened with physiological saline solution. Do not put it in the solution. Keep the container likewise at a low temperature.

Carefully avoid injury to the tissue from any cause. Letting its surface dry during removal from the body, or at any time before fixation, produces **Artifacts.** So also does mechanical manipulation. If forceps must be used, do not pinch the part of which the preparation is to be made. It is better to lift the tissues. Scissors tend to squeeze the tissue, but it is necessary to cut with them in some cases. The ideal way is to cut with a sharp razor blade. This is easy with the liver, kidney, brain and other more or less compact organs, but the sweep of a razor blade tends to draw the tissue and cause displacement, especially when the specimen is heterogeneous, some parts being loose connective tissue, others muscle, others gland, etc. When feasible, cut the tissues into slices and lift them into the fixative. For fixatives that penetrate easily (formalin, Zenker's and Bouin's fluids, etc.) make the slices 4-6 mm. thick. For the poor penetrators, in which osmic acid is the principal ingredient (Bensley's A.O.B., Flemming's fluid, etc.), the slices must be not more than 2 mm. thick. In the case of surface tissues (skin, gastric mucous membrane, bladder wall, etc.) fix a strip, flattened on the surface of a piece of wooden tongue depressor or stiff paper card. A volume of fixative at least 20 times that of the tissue fixed is required.

Agitate the bottle slightly to prevent the tissue from sticking to the bottom and to ensure penetration from all sides.

It may be desirable to inject the fixative *via* a large artery supplying the tissue to be examined. This eliminates mechanical injury to the tissue before fixation, preserves gross form relations better and is suggested when sections are required of large specimens. Before injecting the fixative wash out some of the blood by **Perfusion** with physiological salt solution, or at least let the blood drain out from the veins, because, if all is left in, it may clog the arteries and block the entry of the fixative. After fixation by vascular injection it is customary to cut, with a razor blade, suitable slices and to continue the fixation by immersion. Obviously such tissues should not be employed for microchemical analyses because there is a danger of washing out chemical substances. Clearly, also, the speed of fixation depends upon the degree of vascularity. For avascular tissues such as epidermis, cornea and cartilage fixation by injection is not recommended.

After the tissues have hardened a little by immersion in or injection with the fixative, it may be helpful to remove them from the fixative and trim them with a razor blade so that their size and shape will be almost what is needed when they are finally cut into sections. The slices should have smooth upper and lower surfaces including an area which will yield sections that will fit nicely under a 22 x 22 mm. cover glass unless larger covers are to be used. The shape should be rectangular with opposite edges parallel. In general it is well to have two longer parallel edges and two shorter ones, because a square surface is not so convenient to section as an oblong one. However one must bear in mind exactly what one wishes to demonstrate. This making of uneven surfaces smooth does however introduce an experimental error; because, where much is shaved off, the fixation has penetrated less than where little or no tissue has been removed. After trimming return tissues to a fresh supply of fixative. Tissues fixed in poor penetrators should not be trimmed.

The time of fixation depends upon the tissue, the fixative and the purpose in mind. In general, 24 hrs. is suitable. Some fixatives, particularly those containing potassium bichromate and/or osmic acid, are not very stable and for this reason should be renewed. The fixative deteriorates less quickly if the fixation is carried out at a low temperature in an ice box. The speed of fixation is probably also somewhat diminished. The effect of pH on chromium fixatives has been studied by Zirkle (C., Protoplasma, 1928, 4, 201–227). Fixation involving **Decalcification** and **Mordanting** are special cases described under these headings. For choice of fixative see **Fixatives.**

After fixation **Washing** may be necessary, or **Mordanting.** The tissue may be prepared as a **Whole Mount,** or **Frozen Sections** may be made, or it may be dehydrated, cleared and imbedded in **Paraffin** or dehydrated and imbedded in **Celloidin** for **Sectioning.**

**Fixatives.** The number from which to choose is enormous but the number actually employed is comparatively small. **Formalin** unquestionably heads the list as being used for a far greater variety of purposes than any other fixative. It penetrates well and is an excellent preservative. It is the only satisfactory fixative for use before the cutting of frozen sections and as a preliminary to certain microchemical reactions. **Alcohol** comes next in variety of services performed but unfortunately it brings about considerable shrinkage. Both formalin and alcohol are frequently combined with other ingredients.

For routine purposes **Zenker's Fluid,** either alone or with formalin, is perhaps the most popular fixative. Tissues so fixed give better contrasts of acidophilic and basophilic components than are obtained after fixation in formalin or alcohol by themselves. **Bouin's Fluid** is also an excellent fixative for general use and is being employed with increasing frequency. It is particularly advocated by dermatologists. **Regaud's Fluid** is the fixative of choice for mitochondria because it penetrates so much better than **Osmic Acid** containing fixatives. No important new fixatives have recently been devised.

In making the selection one is naturally guided by data concerning the structures which it is desired to demonstrate (see **Nerve Endings, Mitochondria,** etc.) or the substances to be revealed (**Lead, Copper, Oxidases, Lipids,** etc.) or the techniques that seem best adapted to the purpose in mind (**Mallory's Connective Tissue** stain, **Weigert's Method,** etc.). Some of the more important fixatives are listed, further data being given under each heading.

| | |
|---|---|
| Acetic osmic bichromate | Basic lead acetate |
| Alcohol (ethyl) | Bouin |
| Allen | Cadmium chloride |
| Barium chloride and formalin | Carnoy |
| | Carnoy-Lebrunn |
| | Champy |

Chloral hydrate
Destin
Diaphanol
Dioxan
Downey
Erlicki
Ferric chloride-osmic acid
Flemming
Formalin
Formalin-Zenker
Giemsa
Gilson
Helly
Hermann
Hischler
Kleinenberg
Kolatchew
Lactophenol
Lillie
Mann
Marchi
Maximow
Mercuric chloride
Methyl alcohol
Muller
Orth
Osmic acid
Parabenzoquinone
Perenyi
Petrunkevitch
Regaud
Rabl
Schandinn
Silver nitrate
Susa
Tellyesnicky
Weigert
Van Gehuchten
Zenker
Zweibaum

**Flagella.** 1. Of *bacteria.* Loeffler's stain. Mordant in fresh 20% aq. tannic acid, 10 cc.; sat. aq. ferrous sulphate, 5 cc.; 3–5% basic fuchsin in 95% alc., 1 cc. gently heated, 1 min. Rinse in water stain with slight heat in **Carbol Fuchsin** 1 min. wash and dry. For other flagella stains *see* discussion in McClung (pp. 143–145) and Shunk, I. V., J. Bact., 1920, **5**, 181; Galli-Valerio, B., Centralbl. f. Bakt. Orig., 1915, **76**, 233; Gray, P. H., J. Bact., 1926, **12**, 273. See technique for darkfield study of flagella (Pijper, A., J. Path. & Bact., 1938, **47**, 1–17).

2. Of *erythrocytes* (Oliver, W. W., J. Inf. Dis., 1934, **55**, 266–270). Add 1 mg. hirudin to 2–3 cc. sterile Ringer's solution in small, sterile test tube. Draw up about 0.5 cc. into a sterile Pasteur pipette fitted with rubber bulb. Apply to drop fresh normal blood from finger. Suck up quickly into pipette and expel into test tube. Incubate at 37 °C. 40–50 min. which promotes flagella production. Add small drop to clean slide held at 40° angle. After the drop has run down slide, let dry completely in horizontal position at room temperature. Mordant in fresh 10% aq. tannic acid, 50 cc.; sat. aq. ferrous sulphate, 25 cc. and sat. alc. basic fuchsin, 5 cc. which is poured on slide and warmed slightly 20 min. Wash thoroughly in running tap water and dry. Flood with fresh Ziehl-Neelsen (1 gm. fuchsin, 10 cc. alcohol + 90 cc. 5% aq. phenol acid) 20 min. not warmed. Wash carefully in running water, blot dry and examine with oil immersion. It will be helpful to examine Oliver's illustrations.

**Flavins** under fluorescence microscope show green fluorescence in liver tissue. See **Riboflavin.**

**Fleas,** see method of double imbedding for (Lee, p. 598).

**Flemming's Fluid.** *Weak:* 0.25% chromic acid, 0.1% osmic acid and 0.1% glacial acetic acid in aq. dest. *Strong:* 1% chromic acid, 15 cc.; 2% osmic acid, 4 cc.; glacial acetic acid, 1 cc. These are classic fixatives now not much used. The Bensleys (p. 45) advocate same ingredients differently made up. A: 1% aq. chromic acid, 11 parts; glacial acetic acid, 1 part; and aq. dest., 4 parts. B: 2% osmic acid in 1% aq. chromic acid. Immediately before use, mix 4 parts of A with 1 part of B and employ a volume ten times that of the tissue. Fix 2–72 hrs. and wash in water 24 hrs. See **Safranin-Gentian Violet** and **Orange G** method, **Mitosis, Benda's Method.**

**Fluoran Derivatives.** As explained by Conn (p. 144) fluoran is not a dye but a product of phthalic anhydride containing a xanthene ring and a lactone ring with introduced hydroxyl groups and halogen atoms in particular positions. Examples: eosin B and Y, erythrosin bluish and yellowish, ethyl eosin, fluorescein, mercurochrome 220, methyl eosin, phloxine, phloxine B, rose bengal.

**Fluorescein** (CI, 766) is simplest fluoran dye. It stains very poorly but is highly fluorescent. Its sodium salt is called uranin.

**Fluorescence Microscopy.** Details provided by Dr. W. L. Simpson of The Barnard Free Skin and Cancer Hospital:

Fluorescence is the property, possessed by many substances, of converting short wavelengths of light into longer wavelengths. In the field of microscopy those structures and substances are of most interest that convert ultraviolet light into light of the visible spectrum, since it is only these substances that can be observed directly. Though fluorescence microscopes designed for this type of observation have been available commercially for 30 years, their use has been limited until recently by their relatively high cost and by the apparent failure of biologists to appreciate the possibilities of this type of observation. Recent technological developments in the glass and electric lamp industries now make it possible to assemble an apparatus for fluorescence microscopy at a cost well within the budget of most laboratories. Evidence of heightened interest in this field is found in the numerous papers concerning fluorescence microscopy within the past 10 years. Although several reviews of the subject already exist (Haitinger, M., Fluorescenz-Mikroscopie, Akademische Verlagsgesellschift, Leipzig, 1938; Hamperl, H., Virchows Arch. f. path.

Anat., 1934, **292**, 1–51; Sutro, C. J., Arch. Path., 1936, **22**, 109–112; and McClung's Handbook of Microscopical Technique, New York, Paul B. Hoeber Inc., 1937), the technique will be described as it can be used with an assembly of low cost apparatus available in the United States at the present time.

*Apparatus required:*

1. An intense source of ultraviolet light that is rich especially in the region from 300 to 400 millimicrons. Certain electric arcs using electrodes of special metal alloys (the Haitinger Arc, C. Reichert—Vienna) have been developed for this purpose. More easily available, low in cost, and having an intense output in the desired region, are the medium pressure mercury vapor arcs in capillary quartz tubes (the A H 4 lamp of the General Electric Company or Westinghouse Electric Co. and lamps made by Hanovia Chemical Co., etc.).

2. Filters that remove all or nearly all of the visible light. A considerable selection of glass and liquid filters may be used for this purpose. Since most of the so-called ultraviolet filters pass also a certain amount of red light, supplemental blue filters must be used with them. A solution of copper sulfate in a cell or tube of quartz, or of ultraviolet transmitting glass, is satisfactory and readily available. A combination of Shott glass filters U G 2 and B G 14 are recommended by Jenkins (R., Stain Techn., 1937, **12**, 167–173). Corning Filters #5840, 5860, or 5874 used with a copper sulfate solution are satisfactory in our experience. An entirely liquid filter, using solutions of cobalt sulfate and nickel sulfate, is described by Bäckström (H. L. J., Arkiv. för Kemi. Mineralogi Och Geologi, 1940, **13A**, 1–16).

3. Condensing lenses, if used at all, must be of quartz or ultraviolet transmitting glass.

4. A quartz prism or mirror of polished metal having a high reflecting power for ultraviolet. Aluminum and magnesium-aluminum alloys are best for this. By mounting the microscope and light source horizontally this item can be eliminated.

5. An ordinary microscope that is fitted with a substage condenser of quartz or ultraviolet transmitting glass. Since the ultraviolet light has served its purpose when it has reached the tissue, ordinary glass objectives and eyepieces are used. With some older objectives the balsam of the lenses fluoresces in ultraviolet and causes an unpleasant diffuse light to appear in the microscope that masks the fluorescence of the tissue. This may be eliminated with a darkfield stop that prevents direct rays of ultraviolet light from entering the objective. Newer lenses are free from this fluorescence and may be used without a darkfield stop. This is desirable since it permits the utilization of a greater portion of the light that strikes the condenser. Popper has reported that the fluorescence of **Vitamin A** can be observed with an ordinary microscope with glass condenser. Ordinary optical glass transmits sufficiently far into the near ultraviolet that this type of apparatus might be successfully used for strongly fluorescent substances.

6. Slides for the specimens of ultraviolet transmitting glass. (Corex D glass slides, obtainable from Corning Glass Co. are suitable.)

7. An eyepiece filter that excludes ultraviolet light with a minimum absorption of visible light. This may be of glass (Leitz ultraviolet protecting filter no. 8574 A, Corning Glass Works filters no. 3389 or 3060) or, simplest and cheapest, a circle of Wratten 2A gelatin filter cut to fit within the eyepiece (the Wratten 2 filter is not suitable since it fluoresces itself in ultraviolet light).

8. Non-fluorescent media for mounting the section to be examined. Medicinal mineral oil, or glycerin is suitable. If immersion lenses are to be used non-fluorescing immersion medium must be employed. Sandlewood oil has been recommended for this purpose.

*Preparation of tissues:* Hamperl (loc. cit.) recommends that tissues for fluorescence examinations be fixed only in a dilute solution of formalin, since metal containing fixatives destroy the fluorescence of some substances. A 5–10% solution of U.S.P. formalin in aq. dest. is ordinarily employed. Tissues should should not be fixed longer than 24 hrs.; certain components of tissue acquire abnormal fluorescence if the time of fixation is prolonged. If fats and other alcohol soluble substances are to be examined, i.e., vitamin A, polycyclic organic carcinogens, etc., frozen sections must be made. If these substances are not of interest, the tissue may be dehydrated, cleared, and imbedded in paraffin in the usual manner. High quality reagents are required, because the impurities found in many organic substances themselves fluoresce. All paraffin must be removed since this too fluoresces. The section can be cleared in anhydrous glycerin or pure medicinal mineral oil. Gelatin and celloidin are not recommended for imbedding because of their fluorescence.

Two types of fluorescence may be pro-

duced in tissues with this type of apparatus. The first is that seen in tissues that have been subjected to no special treatment and is due to the presence of fluorescent substances in the tissues themselves. This is termed "primary" fluorescence or natural fluorescence and is exhibited by many substances found in animal organisms. In most tissues there are present sufficient quantities of these materials to permit the observer to recognize the general structure of the tissue without recourse to stained control sections studied with transmitted visible light. Hamperl (*loc. cit.*) describes, in considerable detail, the natural fluorescence of many human tissues. Jenkins (*loc. cit.*) summarizes the findings in the most common animal tissues. Cornbleet and Popper (T. and H., Arch. Dermat. & Syph., 1942, **46**, 59–65) review the natural fluorescence of human skin. Popper and his coworkers have contributed a series of papers on the fluorescence of vitamin A in animal tissues (Popper, H., J. Mt. Sinai Hosp., 1940, **7**, 119–132. Arch. Path., 1941, **31**, 766–802; Popper, H. and Brenner, S., J. Nutrition, 1942, **23**, 431–443; Popper, H. and Ragins, A. B., Arch. Path., 1941, **32**, 258–271). Simpson and Cramer (W. L. and W., Cancer Research, 1943, in press) have used the method to follow the distribution and persistance of methylcholanthrene in skin.

Another kind of fluorescence is the "secondary" fluorescence that appears in certain components of the tissue after sensitization with dyes and plant extracts. This extends considerably the range of fluorescence microscopy and has been developed chiefly by Haitinger (loc. cit.) in conjunction with Hamperl and Linsbauer. Various fluorescent alkaloids, azo dyes, primulins, auramine, berberine sulfate, chelidonium, rhubarb extracts, etc., are selectively absorbed by certain parts of the cell and cause them to show characteristic fluorescences in ultraviolet light. Such substances are called fluorochromes. Sections of tissue are immersed in such substances for a short period of time before being examined. Examples of the use of these fluorochromes are found in papers by Haitlinger (loc. cit.), Jenkins (loc. cit.), Clark and Perkins (W. M. and M.E., J. Am. Chem. Soc., 1932, **54**, 1228–1248), Lewis (M. R., Arch. f. exp. Zellf., 1935, **17**, 96–105) and Popper (H., J. Mt. Sinai Hosp., 1940, **7**, 119–132). See **Vitamin A, Tubercle Bacillus, Cell Injury, Uranium, Porphyrins,** etc.

**Fluorescence Spectra.** The technique in some detail is described for 3:4-Benzpyrene by Hieger, I., Am. J. Cancer, 1937, **29**, 705–714 who thinks that the photograph of the spectra can well be studied by simple visual examination.

**Fluorescent Blue,** see **Resorcin Blue.**

**Fluorescent X.** A special type of reduced neutral red (Clark, W. M. and Perkins, M. E., J. Am. Chem. Soc., 1932, **54**, 1228–1248) employed for tissue cultures (Lewis, M. R., Arch. f. exp. Zelf., 1935, **17**, 96–105).

**Fluorochromes.** See **Fluorescence microscopy.**

**Foot's Methods.** 1. Rapid silver impregnation of *reticular fibers* (Foot, W. C., J. Tech. Meth., 1929, **12**, 117–119). Fix in 10% formalin (not necessarily neutral), Zenker's, Bouin's or Helly's fluids, 24 hrs. Make paraffin sections. Remove mercury, if present with iodine. Treat with 0.25% potassium permanganate, 5 min. and 5% oxalic acid, 10 min. Wash in aq. dest. Impregnate 15 min. in following silver solution at 50°C.: Add 40 cc. 5% aq. $Na_2CO_3$ to 10 cc. 10% aq. $AgNO_3$. Let precipitate settle. Decant supernatant fluid. Make up to 50 cc. with aq. dest. Shake, repeat settling and decanting. Dissolve ppt. in just sufficient $NH_4OH$, added drop by drop, to almost completely dissolve it leaving a few gray granules. Heat to steaming to drive off excess $NH_3$ and cool to 50°C. Reduce in 1% formalin 2 min. Wash in tap water. Tone 2 min. in 0.2% aq. gold chloride. Wash. Tone in 5% aq. sodium thiosulphate. Counterstain with hematoxylin-Van Gieson. Reticulum, black; collagenic fibers, vermillion; cytoplasm, yellow; and nuclei, brown.

(2). Silver method for *nerve fibers* in paraffin sections (Foot, N. C., Am. J. Path., 1932, 8, 769–775). This is a modification of Cajal's technique. Fix in fresh **Carnoy's Fluid** for 24 hrs. Transfer to absolute alcohol for 1–2 hrs., clear in chloroform and imbed in paraffin. Remove paraffin from sections in usual way. Treat with 2 parts pyridine and 1 part glycerol for 1–12 hrs. Wash in 95% alcohol and then in aq. dest. to remove most of pyridine. A trace remaining does no harm. Immerse in 10% aq. silver nitrate at 37°C. for 12 hrs. or so covering container to avoid evaporation. Wash in 2 changes aq. dest. Place in 5% aq. neutral formalin containing 0.5% pyrogallol in which sections become yellow-brown, 20 min. Wash under tap. Tone in 1:500 aq. gold chloride, 5 min. (Nuclear precision is improved and glacial impregnation is made less intense if 2% glacial acetic is added to gold solution). Then place in 2% oxalic acid containing 1% neutral

formalin for 5 min. Wash at the tap and transfer to 5% aq. sodium thiosulphate for 5 min. Finally wash again in running water, dehydrate, clear and mount. Foot also recommends Rogers' technique practically as given by him (Rogers, W. M., Anat. Rec., 1931, **49**, 81–85) The idea of intensifying the gold toning with oxalic acid he credits to Laidlaw, G. F., Am. J. Path., 1929, **5**, 239–247. See general remarks on **Silver Methods.**

**Formaldehyde** is a gas (HCOH) soluble to 40% in water producing a solution which is termed commercial formalin or formol. See **Formalin.**

**Formalin** (Formol) is a 37% aq. solution of the gas, formaldehyde. Solutions employed as fixatives and preservatives are made in terms of the percentage of formalin, not of formaldehyde. Thus, a 10% solution of formalin (formol) is by convention 10 cc. of formalin plus 90 cc. of water. It is not however 10% formaldehyde. (Obviously to dilute 10 cc. conc. hydrochloric acid with 90 cc. of water would not give 10% hydrochloric acid because conc. hydrochloric acid is not 100% so that this practice cannot be extended.) Formerly it was necessary to neutralize commercial formalin in various ways, and it still is for special purposes. The best way to obtain neutral formalin is to distil. Atkins (Lee, p. 61) advises addition of borax to the diluted formalin until it shows a good red color with phenolphthalein or slaty blue with thymol blue. Others simply add a little calcium, sodium, magnesium or even lithium carbonate. Obviously the addition of such minerals greatly complicates the problem when formalin is employed with alcohol as a fixative preliminary to microincineration. Unless neutral formalin is specified and the manner of neutralization, it is best simply to use the fairly pure product which now can readily be obtained. Experiments by Davenport, H. A., Stain Techn., 1934, **9**, 49–52 show that as a neurological fixative slightly acid formalin is somewhat better than neutral formalin. A few of the many demands for formalin as a fixative will be found under:

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|---|---|
| Alizarin red S | Buzaglo |
| Alveolar pores | Cajal's brom-formol |
| Amyloid | Cartilage |
| Argentaffine cells | Chitin |
| Arsenic | Chloride |
| Articular nerve terminals | Chorioallantoic membrane |
| Bile pigments | Christeller-Koyama |
| Bismuth | Chromaffin reaction |
| Bodian | Color preservation |
| Bone | Connective tissue cells |
| Dopa oxidase | Mallory's connective tissue stain |
| Fatty acids | Microglia |
| Fluorescence microscopy | Mucus |
| Foot | Nile blue sulphate |
| Frozen sections | Perdrau |
| Giemsa staining | Pia mater |
| Glia staining | Romieu |
| Gomori | Schultz cholesterol |
| Gordon | Sebaceous glands |
| Grieves | Silver citrate |
| Johnson's neutral red | Smith-Dietrich |
| Kinney | Spirochetes |
| Krajian | Vorhoeff |
| Liebermann - Burchardt | Weigert-Pal |
| Madder staining | Weil |
| | Wilder |

In combination with other reagents formalin is also a good fixative cf. **Formalin-Zenker** in which formalin is substituted for acetic acid, **Bouin, Regaud's Fluid** and many others. Since, however, formalin is a strong reducing agent mixtures of which it is a part are unstable so that it must be added immediately before use. As Mallory (p. 40) points out, formalin also has certain disadvantages. It is inferior to alcohol as a preservative for iron and other pigments. It often changes the color of bile concretions from yellow to green and it may produce in the tissues a troublesome brown-black finely divided crystalline precipitate from laked hemoglobin. He advises removal of this precipitate by treating sections for 30 min. in 75% alcohol, 200 cc.; plus 25–28% ammonia water, 1 cc. (Schridde's method), or for 10 min. in 80% alcohol, 100 cc. plus 1% aq. potassium hydroxide, 1 cc. (Verocay's method) after either of which they are washed thoroughly in water before placing in 80% alcohol and staining. When employed as a preservative concentration of formalin should be 4%.

**Formalin-Alcohol,** see **Alcohol-Formalin.**

**Formalin-Zenker.** Zenker's fluid modified by substituting 5% formalin in place of the 5% acetic acid. It is also known as Helly's fluid and Zenker-formol. This is one of the three major routine fixatives the others being Zenker and Bouin. See **Acid Fast Bacilli, Alveolar Pores, Arteries, Basal Bodies, Brazilin-Wasserblau, Mucus, Goodpasture's Method, Methyl-Green Pyronin.** In some cases 10% formalin is inserted instead of 5%.

**Formalose** see **Formalin.**

**Formamide** of Eastman Kodak Co. is a substance, called a "modifier", which when added in 10% to 50% alcohol improves fixation and staining of peripheral nerve (Bank, E. W. and Davenport, H. A., Stain Tech., 1940, **15**, 9–14).

**Formol** is a synonym for formalin.

**Formol-Müller.** This is 1 part of formol to 10 parts Müller's fluid.

**Formol-Nitric** fixative. 3 parts 10% formalin and 1 part 10% nitric acid. This has, according to McClung, proved very valuable for chick embryos.

**Fractures.** Vital staining with **Alizarin Red S** (Schour, et al., J. Dent. Res., 1941, **20**, 411–418).

**Frozen Sections.** These are of great value when preparations must quickly be made and when methods of alcoholic dehydration before sectioning are contraindicated. They are specified elsewhere in this book under several headings including:

| | |
|---|---|
| Amyloid | Lipase |
| Cholesterol | Lipids |
| Digitonine reaction | Microglia |
| Dopa oxidase | Millon's reaction |
| Gold | Oxidase |
| Indigo-carmine | Pepsin |
| Krajian's Congo stain | Spirocheta pallida |
| Liebermann-Burchardt | Urease |

To make the sections take recently excised still living tissue, or better fresh tissue fixed for about 30 min. in 10% formalin. First freeze a little water on the block of a freezing microtome. Then add the tissue and freeze it too plus a drop more of water. Allow block to thaw to optimum consistency, cut sections 15–50$\mu$ thick as desired, and remove them with a camel's hair brush from the microtome knife to formalin, water or physiological saline. When many are required, it may be necessary to freeze several times as the tissue becomes too soft. If thinner sections are wanted resort to **Gelatin Imbedding** before sectioning.

For quick staining Thibaudeau, A. A., J. Lab. & Clin. Med., 1933, **19**, 204–209 advises that sections of formalin fixed tissue be stained in Harris' hematoxylin 5–15 sec., rinsed in aq. dest., blued in aq. dest. + few drops $NH_4OH$, passed up through 70, 85 and 95% alcohol, counterstained in absolute alcohol and eosin (5 sec.), cleared in carbol xylol, blotted with filter paper and mounted in balsam. Proescher, F., Proc. Soc. Exp. Biol. and Med., 1933, **31**, 79–81 recommends pinacyanol as giving excellent color contrasts. Perhaps the simplest method advised by the Bensleys (p. 138) is to stain the sections in **Goodpasture's Acid Polychrome Methylene Blue** (which see) 1 min. or longer, wash and mount in aq. dest. This colors nuclei dark purple and connective tissue bright rose red. But methylene blue is less permanent than hematoxylin.

For *reticular* and *collagenic fibers* in frozen sections proceed as follows (Krajian, A. A., Arch. Path., 1933, **16**, 376–378): After fixation in 10% formalin, cut sections 5–10 microns and wash in aq. dest. Then 10% aq. $NH_4OH$ at 60°C., 15 min. Wash in 3 changes aq. dest. and place in 0.3% $KMnO_4$ for 5 min. Rinse in aq. dest., decolorize in 1.5% oxalic acid until brown color has entirely disappeared. Wash 4–5 times in aq. dest. and soak in 5% $AgNO_3$ at 60°C. for 1 hr. Wash twice in aq. dest. Transfer to ammoniacal silver sol. (to make add 6 drops 10% NaOH to 8 cc. 10% $AgNO_3$. Then add freshly prepared 10% $NH_4OH$ drop by drop until almost entirely clear. Dilute to 28 cc. with aq. dest.) 16 min. at 60°C. Wash 3 times quickly in aq. dest. Change to 30 cc. formaldehyde + 70 cc. aq. dest. 1–3 min. at 60°C. Wash in tap water. Mount on slide. Dehydrate with a little absolute alcohol and blot into position. Dehydrate more, blot, 3 changes equal parts anilin oil and xylol, xylol, balsam. Reticular fibers jet black, collagenic ones dark brown.

For serial sections of brain (Marshall, W. H., Stain Tech., 1940, **15**, 133–138) fix slices 24 hours or longer in 10 or 15% formalin and then treat them with a 20–30% alcohol or in 15% formalin in 20% alcohol. The object of the alcohol treatment is to avoid formation of hard and brittle ice crystals which fracture the sections as they are made. Cut tissue into blocks about 1.0 cm. thick. Place on a $CO_2$ ice freezing disc which has been covered by a piece of wet blotting paper. (In our laboratory we use a regular $CO_2$ gas freezing disc which has been adapted to a precision sliding microtome.) Freeze the block of tissue slowly throughout. The proper degree of freezing depends on the thickness of the sections to be cut. Marshall recommends a paraffin knife, 20–30° angle with block, knife set in a line perpendicular to the direction of motion. Remove cut sections by a camel's hair brush to 50% alcohol and keep them in serial order. Mount sections serially on slides coated with **Albumen-Glycerin.** Smooth out wrinkles and flatten sections by gentle pressure with blotting paper moistened with 50% alcohol. Remove slides to a 38°C. oven for 4–6 hrs. when they are ready for staining. (In some cases it may be inadvisable to press the sections flat upon the slide. Thin sections require less drying than thick ones. In any case until one has gained confidence in the use of the technique, the sections should

be observed at intervals in the 38°C. oven. At the least sign of excessive drying (whitening of parts of the section) the sections should at once be removed to the stain). The **Cresyl Violet** method of Tress and Tress is recommended.

**Fuchsin NB,** see **New Fuchsin.**

**Fuchsin S, SN, SS, ST** or **S III,** see **Acid Fuchsin.**

**Fungi.** Data contributed by Dr. Morris Moore of The Barnard Free Skin and Cancer Hospital.

1. *Skin scrapings and hair.* The usual method is to mount the material in an alkali—either sodium hydroxide (NaOH) or potassium hydroxide (KOH). The latter is preferable and should be used in a 10–30% solution. For rapid work 40% is employed but this tends to swell and disintegrate the fungi. A weak solution takes longer to clear the skin. The skin usually clears in 5 min. to 2 hrs. in concentrations of 10–30%. A little heat helps. Use subdued light in order to avoid high lights. The fungus is clearly discernible against the irregular nondescript background of skin which is usually clear. Dip infected hairs taken from scalps, particularly those that are oily, in ether or in alcohol (absolute alcohol is preferable to 95%) for a moment in order to get rid of the oil which often simulates spores in shape and size.

Adamson (H. G., Brit. J. Dermat., 1895, 7, 201–211, 237–244) has recommended clearing with 5–10% KOH and staining by the Gram method. Chalmers and Marshall (A. J. and A., J. Trop. Med. Hyg., 1914, 17, 256–265, 289–291) suggest soaking scales in 40% KOH for some hours in a watch glass in an incubator at 40°C. Transfer specimens to watch glass containing 15% alcohol for 30 min., remove to slide, allow alcohol to evaporate and dry over flame; stain with **Anilin-Gentian Violet** for 20 min. Treat with **Gram's Iodine** for 3 min.; decolorize with anilin oil, 30 min.; stain in concentrated alcoholic eosin, 1 min.; wash off eosin with anilin oil or clove oil; treat with xylol and mount in balsam.

Priestley (H., Med. J. Australia, 1917, 2, 471–475) recommends lactophenol (lactic acid, 1 part; phenol, 1 part; glycerol, 2 parts, aq. dest., 1 part) for clearing instead of 40% KOH; or chloral hydrate crystals, 2 parts; lactic acid, 1 part; phenol crystals, 1 part, may be used. For thick material Langeron suggests: chloral hydrate crystals, 40 gm.; phenol crystals, 40 gm.; lactic acid (U.S.P.), 20 gm.; and sodium salicylate, 10 gm. Slight heat facilitates clearing. To stain, Priestley recommends treatment with chloroform to remove the fat; boiling, 2–3 min., with formic acid; washing for a few minutes in water and staining with Sahli's methylene blue: after which the tissue is to be washed, differentiated with alcohol if necessary, dehydrated, cleared and mounted in balsam.

Bachman (R. W., Arch. Dermat. & Syph., 1920, 1, 50–54) recommends the following procedure: Place scrapings in a drop of water on a cover slip, tease thoroughly with a dissecting needle, dry over a flame but do not scorch. Stain for 2 min.; decolorize in 95% alcohol, 15–30 sec.; immerse in aq. dest., 15–30 sec.; pour off excess, dry by heat, and mount in balsam. Spores and mycelium, blue; scrapings, yellow. The dye is sat. alc. gentian violet, 2.5 parts; aq. dest., 17.5 parts; orange G solution, 9 parts; acetic acid, 1 part; 95% alc., 5 parts. The orange G solution is orange G, 2 parts; 95% alc., 20 parts; water, 80 parts. Decolorize with 10–20% KOH.

The hydroxide method of examination is simple and often rapid, but unless used by one familiar with it the results may be misleading. There is danger of confusion with structures which Becker and Ritchie (J. W. and E. B., Arch. Dermat. & Syph., 1940, 22, 790–802) have indicated as resembling yeast cells. These artifacts may be removed by treating the material progressively with absolute alcohol, ether, absolute and 95% alcohol. They have been termed 'mosaic fungus' and have been reported by Greenwood and Rockwood (A.M. and E. M., Arch. Dermat. & Syph., 1930, 21, 96–107) as degenerate fungi. In fact they are cholesterol crystals. The use of dyes eliminates in great measure such artifacts. However, the use of dyes is not practical with thick sections for which recourse must be had to the hydroxide method.

When the scrapings or scales are thin, or when sputum, pus or exudate are examined, a 1% aq. methylene blue and glycerin can be used as follows: One drop of the 1% solution of methylene blue is placed on a clean slide and the material is stirred within it, allowed to stand for approximately 2 min. when a clean cover slip is placed over the mixture and pressed down to flatten out the material and to express the excess solution. The superfluous stain is taken up by filter paper. A drop of glycerin is then placed along one edge of the cover slip and allowed to seep under, displacing the stain and giving a clear background to the stained material. The fungus appears bright blue.

The lactophenol-cotton blue technique was developed in the French laboratories using the formula of Amann (J., Zeit. Wiss. Mikr., 1896, **13**,18–21). Lactophenol consists of phenol crystals, 20 gm.; glycerin, 40 gm.; lactic acid, 20 gm. and aq. dest., 20 gm. Cotton blue (anilin blue, China blue) is a mixture of the trisulphonates of tri-phenyl pararosanilin (C.I. 706) and of di-phenyl rosanilin. Place a drop of the cotton blue (0.5% aq.) on the slide; stir up the material within it and allow to stand for about 2 min. Add cover slip and press down to squeeze out any excess dye, which is taken up by filter paper. Add a drop of lactophenol to the edge of cover slip and allow it to replace the cotton blue which dries out. The stain may be rapidly replaced by holding a bit of filter paper at the edge of the cover slip opposite the lactophenol. The cell wall stains lightly as compared with the darkly colored central portion of the fungus. The tissue elements also stain light blue.

Swartz and Conant (J. H. and N. F., Arch. Dermat. & Syph., 1936, **33**, 291–305) have modified the lactophenol and cotton blue procedure. First put a few scrapings in 5% aq. potassium hydroxide, heat somewhat and wash in water. Place material in a drop of the combined cotton blue (0.5%) and lactophenol. The fungi stain a darker blue than the tissue cells.

Schubert M., Dermat. Wchnschr., 1937, **105**, 1025–1029) has modified the Swartz-Conant technique. Soak the scales in 2% KOH for 30 min. or until they appear glassy and then wash in aq. dest. 2–10 hrs. Transfer small particles to a slide and add 1 or 2 drops of following stain: cotton blue, 0.25 gm.; lactic acid, 10 gm.; phenol crystals, 10 gm.; and aq. dest., 20 gm. The fungi appear dark blue while the epidermal cells stain lightly. See also **Berberian's Method.**

2. *Sputum, pus and exudates:* Examine for fungi after mounting directly on a slide after mixing in 20% KOH or on stained smears. The latter are not very satisfactory because smearing tends to disturb the arrangement of the cells but they are useful for detection of mycelium. Many contaminating organisms are generally present in these exudates unless material is secured from fresh lesions opened aseptically. Several examinations may be necessary since the organisms in exudates are seldom numerous. The hydroxide usually dissolves most of the tissue elements and the fungi stand out as refractile bodies. Several of the staining methods employed in the study of hair and scrapings may be used. Of these, the methylene blue and glycerin method is best but the lactophenol-cotton blue technique is likewise advised.

3. *Vesicles, blister fluid, spinal fluid or urine:* These can also be directly examined. But vesicle, or blister, fluid yields only a small amount of material and for best results, the methylene blue-glycerin method or the lactophenol-cotton blue technique is advised. Urine, or spinal fluid, should be concentrated by centrifugation before examination. The same staining procedures are advocated. See **Blastomycosis.**

4. *Skin:* Unna, Jr. (P., Dermat. Wchnschr., 1929, **88**, 314–321) advises the following modification of the Pappenheim-Unna, Sr. method for staining fungi in skin. Fix in absolute alcohol, then run through the alcohols to xylol and imbed in paraffin. Cut sections at 10$\mu$, stain with pyronine-methyl green (pyronine, 9 parts; methyl green, 1 part; 96% alcohol, 90 parts; glycerol, 100 cc.; 0.5% phenol to make 1000 cc.), 5–10 sec.; rinse in water; dry with absolute alcohol; and mount in balsam. Fungi, rubin red; leukocytes, green to blue green; nuclei of cells of basal horny layer of the epidermis, red.

Fungi in tissue can be easily stained by **Iron-Hematoxylin** and eosin. The fungous elements take the hematoxylin stain nicely, although some difficulty may be encountered in distinguishing spherical cells or spores from tissue elements. The **Gram** method of staining for bacteria has been used with a measurable amount of success since fungi are, in general, gram-positive.

Unna's **Alkaline Methylene Blue** (Unna, P., Monatsh. f. prakt. Dermat., 1891, **13**, 225–237, 286–311), although recommended for staining plasma cells and as a general stain in combination with phloxine or eosin, has been especially adapted for staining organisms in the stratum corneum. It consists of methylene blue, 1 gm.; potassium carbonate, 1 gm.; and aq. dest., 100 cc. The solution stains better after ripening for a week or two and should be diluted 1 to 10 or 1 to 5 before use.

Malcolm Morris (Mallory, F. B. and Wright, J. H., Pathological Technique, Philadelphia and London, 1924, p. 175) in staining various parasites of the skin, avoids the use of potassium hydrate. Place skin in ether, or in 1:1 alcohol-ether; stain for 5–30 min. in 5% gentian violet in 70% alcohol. Then pass through iodine solution, 1 min.; anilin,

or anilin plus 2–4 drops of nitric acid; anilin; and xylol (2 changes) to balsam.

5. *Other tissues:* A number of methods listed for staining **Bacteria** in tissue can be used successfully for fungi. **Mallory's Connective Tissue** stain is good for *Cryptococcus hominis* in brain tissue. Fungus cells, red; thick mucoid capsules, light blue. The **Gram-Weigert** staining method is also excellent. Organisms, deep violet; nuclei, blue to violet; connective tissue, red.

Actinomyces in sections may be stained successfully with **Alum-Hematoxylin** followed by strong eosin. Mallory (p. 279) lists 2 methods of which the following gives good results with paraffin sections of formalin or Zenker fixed tissue. Stain in alum-hematoxylin, 3–5 min.; wash in water; stain in a 2.5% aq. phloxine or 5% aq. eosin, 15 min. in the paraffin oven; wash in water; stain in **Anilin Crystal Violet** (try Stirling's), 5–15 min.; wash in water; treat with **Gram's Iodine** solution, 1 min.; wash in water and blot with filter paper; differentiate in several changes of anilin until no more color comes off; rinse in several changes of xylol and mount in balsam. The branched organisms stain blue while the hyaline sheaths ("clubs") become pink to red.

After the fungi have been successfully cultivated on the various mediums recommended (Moore, M., Arch. Dermat. & Syph., 1936, **34**, 880–886) they can be examined microscopically by transferring part of the growth with a sterile platinum or nichrome wire to a clean slide. This should be done gently to avoid destruction of the fungous growth. The fungus is teased apart gently in one of several fluids such as water, alcohol, alcohol and glycerine (equal parts) or other mounting fluids. Water has a high surface tension and causes disruption of the growth; while alcohol evaporates rapidly and must be replaced. The following solution serves well: 2% potassium acetate, 50 cc.; glycerin, 20 cc.; and 95% alcohol, 30 cc. The preparation is examined with reduced light. The preparations may be stained using one of the several methods listed: lactophenol-cotton blue; methylene blue-glycerin; or Giemsa-glycerin. See **Chorioallantoic Membrane, Actinomycetes.**

**Fuscin** (L. *fuscus*, dusky), a dusky pigment of retinal epithelium usually present in crystalline formations made up of albuminous cores, which determine their shape, plus the adsorbed fuscin material. A relationship to melanin is debated but the pigment is very resistant to chemical attack. It can, however, be bleached completely when exposed to light *in vitro*. For details see Arey, L. B. in Cowdry's Special Cytology, 1932, **3**, 1218.

**Gallocyanin** (CI, 883)—alizarin blue RBN, chrom blue GCB, fast violet—A basic oxazin dye which is an excellent stain for nuclei and Nissl substance (Einarson, L., Am. J. Path., 1932, **8**, 295–307). This method is for celloidin sections and has achieved considerable popularity. Almost any good fixative appears to be satisfactory. The author used 96% alcohol, Zenker's fluid, neutral formalin 1 part + 4 parts aq. dest. and several others. He suggests double imbedding first in celloidin followed by soft paraffin (see **P térfi**) but in this laboratory the usual method of celloidin imbedding is used. To make the stain, dissolve 10 gms. chrome alum ($K_2SO_4 \cdot Cr_2SO_4 \cdot 24H_2O$) in 200 cc. aq. dest. Add 0.3 gms. gallocyanin and mix thoroughly. Warm slowly and boil *for 20 min.* Cool gradually and filter. First rinse 50$\mu$ sections in aq. dest. Stain, 12–24 hrs. agitating. Aq. dest., 1 change. 80% alcohol, agitate occasionally. 95% alcohol, 1 hr.; absolute alcohol, 15 min. Half absolute and ether sufficient time to dissolve celloidin. Absolute alcohol enough to remove ether. Transfer to white oil of thyme to clear, carrying over a minimum of alcohol. Toluol a few minutes. Mount in clarite X. *Note:* the oil of thyme comes from Greece and is not at present obtainable. Use cedar oil instead. The above method gives beautifully stained Nissl bodies in thick sections. If thin sections are wanted imbed in hard paraffin.

**Gametocytes** of estivo-autumnal malaria (see **Malaria**). Serlin, N. J. and Lissa, J. R., Am. J. Clin. Path., 1942, **6**, 8 advise the following method when diagnosis depends on finding gametocytes, or malarial pigment, in peripheral blood. Completely evaporate 1 cc. 1% aq. potassium oxalate in a 15 cc. centrifuge tube. Add 10 cc. venipuncture blood. Mix carefully and centrifuge 30 min. at 2,500 R.P.M. Pipette off all but about $\frac{1}{8}$ in. of supernatant plasma. Smear on 2 slides by wiping buffer layer with stick applicator having non-absorbent cotton tip. Stain by Wright's method. Study of Giemsa stained smears by dark field is suggested (Goosmann, C., J. Lab. & Clin. Med., 1935–36, **21**, 421–424).

**Gastric Contents.** Examine microscopically material obtained by stomach tube after

test meal as described by Stitt (p. 753). Look for mucus, epithelial cells, leucocytes, Gram positive bacilli in smears.

**Gastrointestinal Tract.** Immediate fixation is desirable because postmortem changes occur especially quickly. Do not wash first with water but with physiological saline or with the fixative itself. It may be desirable to place the excised pieces, with peritoneal surface down, on wooden tongue depressor or stiff paper. Some flattening is required. The mucous surface must not be allowed to dry. See **Small** and **Large Intestine.**

**Gautheria Oil** used to be employed as a clearing agent. It has been displaced by the artificial oil, methyl salicylate.

**Gelatin-Carmine** injections, see **Carmine Gelatin** injections.

**Gelatin Glue,** method of mounting sections, see **Masson's.**

**Gelatin Imbedding** and **Sectioning.** This is used when sections are required of loose, friable tissues which easily fall apart. Since the imbedding is directly from water, no alcoholic or other dehydration is required. Probably the best method is that of Zwemer (R. L., Anat. Rec., 1933, **57,** 41–44), devised primarily for the study of adrenal lipoids. Wash material fixed in formalin or other fluid in water, 4 hrs. 5% gelatin in incubator at 35–37°C. 24 hrs. 10% gelatin at same temperature, 12–16 hrs. Imbed by placing in 10% gelatin in Petri dish in refrigerator. Cut out blocks of tissue and fix in 10% formalin several hours to make gelatin insoluble in water. In this formalin solution tissues can be preserved indefinitely. Before sectioning rinse block in water and trim. Freeze with $CO_2$ until block is uniformly white. Allow to thaw until knife cuts easily. Sections as thin as 5 microns can be obtained. Float onto slide in aq. dest. Drain off excess water and run a drop or two of 1% gelatin under section. Again drain off excess. After heating in drying oven at 33–37°C. place slide in 10% formalin for 10 min. to fix gelatin. In this formalin solution the mounted sections can be stored. Stain sections in usual way with **Sudan, Nile Blue Sulphate, Osmic Acid, Laidlaw's Silver Method,** and mount in **Glychrogel.**

Wright's method as described by Mallory (p. 34) is much quicker and is recommended for fragmented tissues such as those from curettings. Make a 10% solution of gelatin in warm aq. dest. and while still fluid add 0.5% carbolic acid. Do not overheat. The tissue, unfixed or fixed, preferably in 10% formalin, is "dried" and placed in a small "pool" of gelatin liquified by heat on a slide or in a glass vessel. This is allowed to solidify in the ice box for 2 hrs. or more. If necessary, store gelatin blocks in 10% formalin. Cut out block containing the tissue, freeze and section. Float sections from water onto slide well coated with albumen-glycerin and spread. Remove excess of fluid and cover with piece of thin cigarette paper. Blot with fine filter paper till cigarette paper is partly dry. Cover cigarette paper with equal parts anilin oil and oil of cloves for few seconds. Drain and peel off cigarette paper. Remove oil by washing in 95% alcohol and pass to water when sections are ready for staining. Mallory suggests methods for **Amyloid, Fat** and staining with **Hematoxylin** and **Phloxine** for general purposes.

**Gentian Blue 6B,** see **Spirit Blue.**

**Gentian Violet.** The problem afforded by this dye, like many others, has been attacked by the Stain Commission. The stain thus referred to has no constancy. Originally it was a mixture in about equal parts of dextrin and methyl violet, the latter itself a mixture in widely varying proportions of tetra-, penta- and hexa-methyl pararosanilins. Later were placed on the market methyl violets with and without dextrin and crystal violet (the hexa methyl compound) all under the label of Gentian violet. As Conn (p. 124) advises the term Gentian violet should be eliminated and crystal violet used wherever in the past the former has been specified. See **Neutral Gentian, Methyl Violet, Crystal Violet.**

**Geranine G** (CI, 127). An acid thiazole dye employed in fluorescence studies on account of color imparted by it under ultraviolet illumination (Conn, p. 70).

**Giemsa's** corrosive sublimate fixative. Sat. aq. corrosive sublimate 2 parts, absolute alcohol 1 part.

**Giemsa's Stain.** 1. For *blood* or *bacteria* in *smears.* Fix air dried smears in methyl alcohol in a covered dish 3–4 minutes. Remove and blot dry. Dilute stock solution of Giemsa in proportion of 1 drop to 1 cc. aq. dest. and stain for 15 minutes. Then wash in aq. dest., blot and dry. If a precipitate is formed in the smear by the stain, invert the slide, support both ends, and the stain will adhere like a hanging drop, kept away from the ends by lines ruled in wax or paraffin. The pH of the aq. dest. used to dilute the stain may be altered by adding very dilute acid or alkali. Optimum pH of 6.4 is given by the McJunkin-Haden buffer. This may be used as diluting medium in place of aq.

dest. Usually the azurophile are stained more distinctly and the neutrophile granules less sharply than by Wright's stain. Bacteria and intracellular protozoa are better colored than by Wright's stain. The May-Giemsa, and Jenner-Giemsa and the panchrome stains of Pappenheim are important modifications. They are listed separately. Present situation concerning Giemsa's stain is that American products give equally good results with thin films but the German product appears to be better for thick ones (Conn, H. J., Stain Techn., 1940, **15**, 41–43).

2. For *sections*. Much depends upon the choice of fixative. Formalin, generally employed in 10% solution, acts as a sort of mordant for the blue component so that the blue coloration is particularly strong. Fixation in Regaud's gives good results particularly with Rickettsia, Zenker's fluid is recommended by Wolbach. When this is used it is necessary to remove the mercuric chloride by treating the sections with Lugol's solution. They are then washed in 95% alcohol and the last traces of iodine are extracted by 0.5% aqueous sodium hyposulphite for 10–15 min. The hyposulphite in turn is washed out in running water about 5 min. and rinsing in aq. dest. See Cowdry's colored figures of Rickettsia, J. Exper. Med., 1925, **42**, 231–252. Bouin's fluid (75 cc. saturated aq. picric acid, 25 cc. commercial formalin and 4 cc. glacial acetic acid) is suggested for intracellular protozoa (East Coast fever parasites) by Cowdry and Danks (Parasitology, 1933, **25**, 1–63) because after Giemsa staining it gives the chromatin a desirable purple color (see colored plate). Stain sections placed vertically in staining jars in 1.5 cc. Giemsa's solution plus 50 cc. aq. dest., changed during the first hour, overnight. Differentiate in 95% alcohol, dehydrate quickly in absolute alcohol, clear in xylol and mount in balsam.

If the sections are not blue enough add 1–2 drops 0.5% sodium bicarbonate and 1.5 cc. methyl alcohol to the stain; or remove excess of mordanting potassium bichromate from Zenker fixation by rinsing 1 min. in 1% potassium permanganate followed by 5% oxalic acid 4 min. and thorough washing in aq. dest., or do both. If on the contrary they are too blue mordant in 5% potassium bichromate 15 min., rinse in aq. dest. until no more yellow is removed and stain; or add a little colophonium to the alcohol used in differentiating and dehydrating of the sections, as advised by Wolbach, or again do both.

Usually Giemsa's stain gives satisfactory results without any special precautions. The difficulty is that the colors fade quite rapidly particularly when the balsam is noticeably acid and when the sections are left in direct sunlight. Their period of usefulness can be extended by mounting in cedar oil, used for oil immersion objectives, instead of in balsam. Try **Clarite.** If a variety of fixatives is employed it may be necessary to suit the stain to the fixative by use of buffers, in which case see Lillie, R. D., Stain Techn., 1941, **16**, 1–6.

**Gilson's Fluid.** Nitric acid (sp. gr. 1.456), 15 cc.; acetic acid, 4 cc.; mercuric chloride, 20 gm.; 60% alc., 100 cc.; aq. dest., 880 cc. Used mostly for invertebrates.

**Gilson's Mixture** is equal parts chloroform and cedar oil.

**Gingiva.** Capillaroscopy of (McClung, p. 401). Eosinophile leucocytes in (Orban, B., J. Dent. Res., 1940, **19**, 537–543.)

**Glacial Acetic Acid,** see **Acetic Acid.**

**Gland Cells** contrasted. Endocrine, exocrine, apocrine, merocrine, holocrine, serous, zymogenic and mucous (Cowdry's Histology, p. 257).

**Glia Staining with Anilin Dyes** (Proescher, Fr., Stain Techn., 1934, **9**, 33–38). Fix in 10% formalin or in 90% alcohol followed by formalin. Wash frozen sections, 10–15 microns thick, in aq. dest. Stain in sat. aq. victoria blue B (not filtered but poured off from the undissolved dye), 14–24 hrs. Wash quickly in aq. dest., mount with glycerin-albumen, blot and dry in air. Treat with ultraviolet light 30 min. Pass to N/20 iodine few sec. Remove iodine, blot, dry, destain in xylol-anilin, clear first in clove oil, then xylol, mount in balsam. Glia blue, nerve cells lightly stained, connective tissue metachromatic violet or colorless. Instead of ultraviolet light stained sections can be treated with 0.5% potassium bichromatic for 30 min. In place of victoria blue, methyl violet 2B, ethyl violet or crystal violet can be employed.

**Glomus.** Aortic and carotid, see **Aortic Paraganglion.**

**Glutathione.** Demonstrated by **Nitroprusside Reaction.** Inhibiting factor in **Vitamin C** silver test.

**Glycerides,** see **Neutral Fats.**

**Glycerine.** Much used in histological technique in the making up of stock solutions of hematoxylin, like **Delafield's,** in **Albumen-Glycerin** used for mounting paraffin sections, etc. It serves as an

excellent clearing agent for the walls of large **Arteries** so that the intramural vessels can easily be distinguished by the blood in them. With potassium hydrate it is employed to clear specimens in the demonstration of **Ossification** centers. As a mounting medium for frozen sections glycerin is invaluable. In the form of **Brandt's** glycerin jelly (which see) glycerin is specified in the technique for **Sebaceous Glands** and many other structures. To make Kaiser's glycerin jelly (Mallory, p. 100) soak 40 gms. gelatin in 210 cc. aq. dest. for 2 hrs. Add 250 cc. glycerin, stir and heat gently 10–15 min. Keep in ice box and melt before use. The 5 gms. carbolic acid crystals specified in Kaiser's formula has unfortunately, according to Mallory, a deleterious influence on alum hematoxylin preparations. See also **Glychrogel** and **Lactophenol.**

**Glychrogel,** as a mounting medium for teased preparations, Marchi stained sections, gelatin sections, etc. To make 100 cc. dissolve 0.2 gm. chrome alum (potassium chromium sulphate) in 30 cc. aq. dest. with aid of heat. Add 3 gm. Knox granulated gelatin in 50 cc. hot aq. dest. Add 20 cc. glycerin with constant stirring and warm. When thoroughly mixed add crystal of camphor (Wotton, R. M. and Zwemer, R. L., Stain Techn., 1935, **10,** 21–22). For use in mounting nematodes (Wotton, R. M., Stain Techn., 1937, **12,** 145–146).

**Glycogen,** the 3 chief methods have been critically studied by C. M. Bensley (Stain Techn., 1939, **14,** 47–52). This account follows her presentation. Since glycogen is labile, immediate fixation of very small pieces of tissue (2–3 mm.) and agitation of the fixative are necessary. She recommends 9 parts absolute ethyl alcohol + 1 part commercial formalin (i.e. 37% formaldehyde) neutralized with $MgCO_3$. If desired the alcohol in this fixative can be saturated with picric acid. After fixation for say 24 hrs. wash in absolute alcohol, embed in the usual way in paraffin (carefully avoiding overheating) or in celloidin.

1. *Best's carmine.* Grübler's carminum rubrum optimum or some other good carmine 2 gm., potassium carbonate 1 gm., potassium chloride 5 gm., aq. dest. 60 cc. Boil gently until color darkens, cool and add 20 cc. concentrated ammonia. Allow to ripen 24 hrs. This is stock solution. Mount paraffin sections, bring down to aq. dest. Stain nuclei with hematoxylin as in the H. and E. technique. Transfer to fresh stain (stock solution 10 cc., 15 cc. conc. ammonia and 30 cc. pure methyl alcohol) for 20 min. Rinse in 3 changes methyl alcohol, dehydrate in acetone, clear in toluol and mount in balsam. Glycogen brilliant red.

2. *Iodine* (Gage). Mount paraffin sections as before, being again careful to avoid unnecessary heat, and bring down to water. Lugol's aq. iodine 10–15 min. Blot with filter paper and dry in air. Mount in yellow vaseline as advised by S. H. Gage (J. Comp. Neur., 1917, **27,** 451–465) with minimum of heat. Glycogen reddish brown.

3. *Bauer-Feulgen.* To make Feulgen reagent dissolve 1 gm. basic fuchsin in 100 cc. aq. dest. by heat. Filter while warm and add when cool 20 cc. normal HCl. Add 1 gm. $NaHSO_3$. Allow to rest 24 hrs., when it should be of pale straw yellow color. Treat deparaffinized sections with 4% chromic acid for 1 hr. or with 1% chromic acid over night. After washing in running water 5 min., place in Feulgen reagent 10–15 min. Rinse 1½ min. in each of 3 changes of molecular sol. $NaHSO_3$ 1 part and tap water 19 parts. Wash in running water 10 min. Counterstain nuclei with hematoxylin if desired. Dehydrate, clear and mount in balsam. Glycogen deep reddish violet, nuclei lavender.

*Control.* Prepare at same time some sections of liver rich in glycogen. Because glycogen is quickly removed by salivary digestion, when sample sections are brought down to aq. dest., spit on them and allow to rest 15–30 min. changing saliva several times. Wash thoroughly in water at body temperature to remove mucus and stain by either of the 3 above mentioned techniques. If the material is then absent in such sections and present in other similarly stained and not digested, it is evidently glycogen. Fixation by the freezing and drying method is even better than with the alcohol, picric, formalin mixture because it is quicker and there is less chance for displacement of glycogen in the cells.

See also for glycogen staining of Trachoma inclusions Thygeson, P., Am. J. Path., 1938, **14,** 455–462. Glycogen is immobilized in its natural position within the cells by the Freezing and Drying technique (**Altmann-Gersh**). Compare figures 3 and 4 of Bensley and Gersch (R. R. and I., Anat. Rec., 1933, **57,** 205–215) showing results by this and other methods.

**Glycol Stearate.** As an imbedding medium (Cutler, O. I., Arch. Path., 1935, **20,** 445–446). Pass up through alcohols to equal parts 95% alc. and glycol stearate in incubator at 56°C. 12–24 hrs. Pure

glycol stearate at 56°C. 24 hrs. Imbed as in paraffin.

**Gmelin's** test for bile pigments. On addition of nitric acid containing a little nitrous acid, color changes to green, then red and finally blue observable under microscope.

**Gold,** microchemical detection of: 1. Method of Borchardt. Modified by Michaelis, O., Biochem. Zeit., 1930, **225,** 478–488. Treat sections of formalin or alcohol fixed tissues for 15 min. in a boiling water bath or for 12–24 hrs. at 40°C. with 5% aq. silver nitrate. Remove ppt. from section with 20% aq. nitric acid. Gold appears as black granules (Lison, p. 100).

2. Method of Okkels, H., C. rend. Soc. Biol., 1929, **102,** 1089–1091. Simply produce gold salt in sections by exposing for at least 12 hrs. to sunlight or to ultraviolet lamp for same time (Gauthier-Villars, P., C. rend. Soc. de Biol., 1932, **109,** 197–198). Lison (p. 100) explains that whatever the technique used it is necessary to prove that the black granules are gold by their insolubility in concentrated acids, solubility in aqua regia (equal parts nitric and hydrochloric acids) and solubility in solutions of potassium or sodium cyanide.

3. Method of Roberts, W. J., Bull. d'Hist. Appl., 1935, **12,** 344–361. Fix tissues in 20% neutral formalin or in Bouin's fluid. Avoid fixatives containing a metal. Wash thoroughly in water. Make paraffin or frozen sections. The latter has the advantage of speed. Make 2 solutions: A. Add 2 gm. silver nitrate pure for analysis to 100 cc. 10% gum arabic in the dark immediately before use. B. Add 1 gm. hydroquinone pure to 100 cc. 10% gum arabic the day before use. Take off the frozen sections in aq. dest. Mix 2 cc. A and 2 cc. B, add 1–3 drops 5% citric acid, shake 30 sec. Leave sections in this mixture 5–10 min. Then without first washing plunge into 5% aq. sodium hyposulphite for a few minutes. Wash thoroughly and mount. Gold in cells is covered with black deposit of reduced silver. Said to be more sensitive method than spectrographic analysis. See author's illustrations.

4. A technique for demonstration of gold in abs. alc. or neutral formalin fixed tissues, based upon reaction with *p* - Dimethylaminobenzylidenrhodanin is described by Okamoto, K., Akagi, T. and Mikami, G., Acta. Scholae Med. Univ. Imp. in Kioto, 1939, **22,** 373–381.

**Gold Choride** for nerve endings, see **Graven's** and **Carey's** methods.

**Gold Orange,** see **Orange II.**

**Gold Orange MP,** see **Methyl Orange.**

**Gold Particles.** The particles of gold are held in colloidal state by the protective colloid, sodium lysalbinate, and are employed to stimulate macrophage production by intravenous injections in animals (Simpson, M. J., J. Med. Res., 1922, **43,** 77–144).

**Golgi Apparatus** (reticular material). The following account is partly based on Cowdry's description in McClung (pp. 274–278). While there is so little agreement as to just what the Golgi apparatus is, it is difficult to describe the technique for its demonstration. What may, however, be regarded as the "type structure" was first revealed by Golgi (Arch. di biol., 1898, **19,** 448–453) in nerve cells through fixation in a mixture containing potassium bichromate and osmic acid followed by impregnation with silver. The apparatus appears jet black against a yellowish background. It is a conspicuous structure consisting of an intricate network of anastomosing strands. This network may closely envelop the nucleus, be concentrated to one side of it, or else be scattered rather diffusely throughout the cytoplasm.

In 1902 Kopsch showed that the same material can be blackened by prolonged treatment with 2% osmic acid. On this affinity for both silver and osmium all the modern methods for revealing the Golgi apparatus are based. Few cytological reactions are more fickle and inconstant, but, when after many attempts the technique is successful, convincing and very beautiful preparations result. Unlike the mitochondria, the Golgi apparatus cannot be studied unstained or supravitally colored in the living cell with any degree of satisfaction except perhaps in some plants (Guilliermond, A., Arch. d'Anat. Micro., 1927, **23,** 1–98; see particularly colored plate 1). There is some evidence however that droplets of material stainable with neutral red may be associated topographically with the Golgi apparatus (Cowdry, E. V. and Scott, G. H., Arch. Inst. Pasteur de Tunis, 1928, 233–252; Covell, W. P. and Scott, G. H., Anat. Rec., 1928, **38,** 377–398).

With both silver and osmium methods considerable experimentation is necessary in order to obtain the best results. The factors to be varied are principally the composition of the fixative and impregnating substance and the time during which they are allowed to act. During impregnation it is always advisable to keep the tissues in the dark and instructions as to temperature requirements should be carefully fol-

lowed. When either the silver nitrate or osmic acid becomes blackened it should be renewed. It is important for the beginner to start with the most favorable material. The spinal ganglion cells of young mammals such as the rabbit are perhaps the best for this purpose. The acinous cells of the pancreas are also recommended but are somewhat more difficult to handle. All of the methods of impregnation outlined below frequently bring to light the mitochondria also.

1. *Cajal's uranium nitrate silver method*, Carleton, H. M., J. Roy. Micr. Soc., 1919, 321–328. This is one of many methods devised by Cajal. It is recommended for embryos and young animals. Fix in uranium nitrate, 1 gm.; formalin, 15 cc.; and aq. dest., 100 cc., 8–24 hrs. Wash quickly in aq. dest. 1.5% aq. silver nitrate 24–48 hrs. Rinse in aq. dest. Hydroquinone, 2 gm.; formalin, 6 cc.; aq. dest., 100 cc.; anhydrous sodium sulphate, 0.15 gm., 12 hrs. Wash in aq. dest., dehydrate quickly, clear, imbed, and section.

2. *Da Fano's cobalt nitrate silver method*, Da Fano, C., J. Roy. Micr. Soc., 1920, 157–161. Here the uranium nitrate is replaced by cobalt nitrate. In other respects the technique is similar. Da Fano has, however, so carefully attempted to control troublesome experimental conditions that the various steps are given in detail. Fix in cobalt nitrate, 1 gm.; aq. dest., 100 cc.; formalin, 15 cc.; 6–8 hrs. The formalin need not be neutralized unless it is strongly acid. In the case of embryos and delicate tissues, when shrinkage is to be feared, reduce the formalin to as little as 6 cc. With cartilage and small pieces less than 3 mm. thick, like the organs of mice, shorten the time of fixation to 3 to 4 hrs. Hollow organs, such as the stomach and intestine, had better be placed in the fixing fluid for 1 hr. and then be cut into pieces of convenient size and shape. For the spinal cord, cerebellum and cerebrum of adults, 8–10 hrs. is recommended, but fixation should never exceed 24 hrs. In the case of the testicle, he advises injection of the fixative through the aorta and then immersion in it. Wash quickly in aq. dest. and impregnate in 1.5% aq. silver nitrate 24–48 hrs. The concentration of silver nitrate should be reduced to 1% for very small fragments easily permeable, and be increased to 2% for tissues containing much fat and for the spinal cord. Impregnation is effected at room temperature in a majority of cases. When difficulty is experienced in impregnation the use of an incubator at 36° to 37°C. is advised. Wash rapidly in aq. dest. and cut down the tissues again to a thickness of 2 mm. or less. Reduce in Cajal's hydroquinone mixture, above mentioned, 12–24 hrs. Wash in aq. dest. ½ hr. Cut with a freezing microtome or imbed in paraffin. The Golgi apparatus should be colored dark brown or black against a yellow background. The preparations may be made more permanent by gold toning. Pass to water. Then 0.1–0.2% gold chloride, 2 hrs. Counterstain with **Alum Carmine**, dehydrate, clear and mount.

3. *Kopsch's method*, Kopsch, F., Sitz.-Ber. d. K. Preuss. Akad. d. wiss. Math. Kl., 1902, **40**, 929–935. Immersion of small pieces of tissues in 2% aq. osmic acid for 8–16 days often brings to light the Golgi apparatus but there is considerable shrinkage and the tissues become rather brittle.

4. *Sjovall's modification*, Sjovall, E., Anat. Hefte, 1906, **30**, 261–391. Fix in 10% formalin, 8 hrs. Wash in aq. dest. 2% osmic acid at 35°C., 2 days. Dehydrate, clear, imbed.

5. *Hirschler's modification*, Hirschler, J. Arch. f. mikr. Anat., 1918, **89**, 1–58. Fix in sat. aq. mercuric chloride, 10 cc.; 2% osmic acid, 10 cc., at room temperature 1–3 hrs. Wash in running water then in aq. dest., ½ hr. 2% osmic acid at 25°C., 12–16 days. Wash for 24 hrs. in running water, dehydrate, clear in chloroform and imbed.

6. *Kolatchew's method*, Nassonov, D. N., Arch. f. Mikr. Anat., 1924, **103**, 437–482. Fix in 3% aq. potassium bichromate, 10 cc.; 1% chromic acid, 10 cc.; and 2% osmic acid, 5 cc., 24 hrs. Wash in running water 24 hrs. 2% osmic acid, 40°C., 8 hrs. 3–5 days at 35°C. Wash in aq. dest., dehydrate, clear, and imbed.

7. *Weigert's Mann-Kopsch method* as modified by Gatenby (Lee's Microtomist's Vade-mecum. Ed. 9. Ed. by Gatenby, J. B., and Cowdry, E. V., London, 1928). Fix in Mann's corrosive osmic acid sol. (sat. aq. corrosive sublimate in salt sol., 10 cc.; 1% osmic acid, 10 cc.) ¼–3 hrs. or more. Wash in aq. dest., 15–30 min. 2% osmic acid, room temperature 10–14 days. Wash in running water 2 hrs. or more. Dehydrate, clear, and imbed. In the sections Gatenby was able to extract the blackening step by step with turpentine and thus to considerably improve the preparations.

8. *Ludford's modification*, Ludford, R. J., J. Roy. Micr. Soc., 1926, 107–109 has experimented at length with osmic acid methods and states that his best results have been obtained as follows.

Fix mammalian and avian tissues in Mann's corrosive osmic sol. 18 hrs. Wash in aq. dest., 30 min. 2% osmic acid at 30°C. for 3 days. Water at 30°C. 1 day, dehydrate, clear, imbed in paraffin and section. A useful variant is to fix in same way and wash in aq. dest. Then osmicate at 35°C. for 3 days, first day in 2% osmic, second in 1% and third in 0.5%. Leave in water for 1 day at 35°C. He recommends various counterstains.

The writer prefers the Ludford techniques. See Lee (pp. 313–326) for a critical statement of the problem; also, Owens, H. B. and Bensley, R. R., Anat. Rec., 1929, **44**, 79–109 for a careful study of factors influencing the osmic acid changes and for their ferric chloride osmic method. Before placing any reliance in the Golgi apparatus as an indicator of cellular activity it is essential (1) to make sure that the technique being used brings to light all the Golgi apparatus, not only a part of it and (2) not to mistake either mitochondria or droplets formed from them for parts of the Golgi apparatus particularly when it is in a dispersed condition spread about in the cytoplasm. But when every known precaution is taken the surface and volume of this peculiar structure can be measured quantitatively by means of a special technique in spinal ganglion cells (Covell, W. P., Anat. Rec., **35**, 149, 1927). According to Monne, L., Protoplasma, 1939, **42**, 184–192 it can be demonstrated by polarized light. Tarao, S., Cytologia, 1940, **11**, 261–281 has described a novel method, which involves the digestion of frozen sections with trypsin and coloration of the Golgi apparatus with Nile blue sulphate, the value of which remains to be determined. An account of alterations in hepatomas is of interest (Dalton, A. J. and Edwards, J. E., J. Nat. Cancer Inst., 1942, **2**, 565–575).

**Golgi Cox Method.** For adult nervous system (Dr. J. L. O'Leary, personal communication). Fix pieces 3–6 mm. thick in following fluid: add 20 cc. 5% aq. potassium bichromate to 20 cc. 5% aq. mercuric chloride. Dilute 16 cc. 5% aq. potassium chromate with 40 cc. aq. dest. and add this to the first two. Do not agitate but leave in fixative until scum forms on surface, usually after 1½–2 months. When impregnation is nearly complete, wash rapidly, dehydrate through graded alcohols and imbed in low viscosity celloidin (see **Celloidin Imbedding**). Cut celloidin sections serially at 80 to 120 microns. Arrange in serial order on slides (80% alcohol). Blot sections dry and cover immediately with 1% celloidin. When somewhat dry, bring slides with sections to water. The sections on each slide may thereafter be treated as a unit. Run sections from water into a saturated solution of sodium sulfite. They rapidly turn a yellow gray. Wash over night and dehydrate through graded alcohols to absolute. Coat with the following varnish, applying it repeatedly in thin even layers, and allowing each to dry partially before applying the next (sandarac, 75 gm.; camphor, 15 gm.; turpentine C.P., 30 cc.; oil of lavender, 22.5 cc.; abs. alc., 75 cc.; add castor oil, 7 drops. Mixture dissolves very slowly.) Since sections are somewhat opaque, the varnish must dry for several days until abs. alc. has evaporated.

**Golgi Methods.** Fundamentally these are different from both the Cajal and Bielchowsky techniques which were later developments. They depend upon a preliminary fixation in a potassium bichromate solution often containing formalin and sometimes other substances such as osmic acid. The silver is selective tending to impregnate a few cells completely which become blackened when it is reduced. Except for the occasional demonstration of the **Golgi Apparatus** these methods do not reveal details of the inner structure of nerve cells like **Neurofibrils** and **Nissl Bodies.** They are of great service in the demonstration of many non-nervous tissue components, the parietal cells of the stomach, bile canaliculi of the liver, Rouget or perivascular cells, etc.

**Golgi Method, Quick.** For brains of newborn animals, and of those 1 day to 30 days old. (Dr. J. L. O'Leary, personal communication.) It is essential to determine the age of the animal at which the cell or fiber selected for study is reaching maturity. For example, if new born kittens are chosen, and the area striata is the object of study, the best impregnations of entering fibers are obtained at 12 to 15 days after birth; of short axon cells, at 18 to 21 days; and of pyramids at 21 to 24 days.

Cut slices of brains 3–4 mm. in thickness by quick cuts of a sharp scissors. Fix in: potassium bichromate, 10 gm.; osmic acid, 1 gm.; aq. dest., 330 cc. Time of fixation must be determined for each part of the CNS studied. In general the older the animal, the longer it is. After fixation, blot blocks of tissue on filter paper and transfer to a bottle containing ¾% aq. silver nitrate. After 24 hrs. the reaction is complete. Imbed in celloidin. Subsequent treatment is very important. Place block in

95% alc. for about 5 min., remove and blot dry. Place block on paraffin disc mounted on a block holder in the orientation desired for cutting. Using a hot teasing needle, melt paraffin around the block so as to fasten block to paraffin. Be sure that melted paraffin does not creep up on the block. Use knife at 45° angle to the block. Cut serially 80–100$\mu$. Place each section as cut in order in 95% alc. using Petri dishes. Be sure not to miss first and last section of the block for these are often more valuable than the entire remainder of the block. Using a spatula, transfer to another 95% alc. after 5 min. After another 5 min. transfer to oil of cloves, arranging in serial order, by placing each section as it enters oil of cloves near the edge of the Petri dish so that it adheres to the edge. When all sections are transferred the group will be placed around the circumference of the Petri dish. As the sections start to retract from the edge, begin to arrange them in the usual order for serial sections. After clearing (clove oil 5 to 10 min.) transfer in serial order to slides. Blot off excess of clove oil and apply xylol, blot off xylol similarly and apply a thin layer of Damar, using the drop method. Let the slide dry on an even surface adding more Damar as necessary to keep sections protected.

**Gomori's Method.** Silver impregnation of reticulum, Gomori, G., Am. J. Path., 1937, **13**, 993–1001. Treat deparaffinized sections of formalin fixed material with 0.5–1% aq. potassium permanganate, 1–2 min. Rinse in tap water and decolorize in 1–3% aq. potassium metabisulphite, 1 min. Wash for several minutes in running tap water. 2% aq. iron ammonium sulphate (violet crystals), 1 min. Wash in tap water few minutes and then pass through 2 changes aq. dest. Impregnate for 1 min. in following solution: To 10% aq. silver nitrate add $\frac{1}{8}$ to $\frac{1}{4}$ of its volume of 10% aq. potassium hydroxide. While shaking add strong ammonia drop by drop until ppt. is completely dissolved. Add carefully silver solution drop by drop until resulting ppt. easily disappears on shaking. Finally add equal vol. aq. dest. Can be kept 2 days in stoppered bottle. Rinse in aq. dest., 5–10 sec. Reduce in commercial formalin diluted 5–10 times with tap water. Wash under tap few min. Tone in 0.1–6.2% aq. gold chloride, 10 min. 1–3% aq. potassium metasulphite for 1 min. Fix in 1–2% aq. sodium thiosulphate (hyposulphite) for 1 min. Wash under tap, dehydrate, clear and mount. Reticulum black. Note author's figures of sarcomata. See **Phosphatase.**

**Gonococcus,** methyl green-pyronin stain. To 10 cc. absolute methyl alcohol add 1 gm. methyl green (dye content 60%) and 0.2 gm. pyronin (bluish certified). Add 100 cc. 2% aq. phenol and shake 2 hrs. per day for 2 days in a mechanical shaker. Filter and add 20 cc. glycerin, C.P. to filtrate. Fix smears by passing slides lengthwise through flame 4 or 5 times. Add stain immediately and warm to slight steaming. Wash off stain 20–50 sec. Dry and examine. Gonococci, deep red; other bacteria except these of Neisseria group pale purplish or barely noticeable; nuclei of pus cells green in soft pink or rose cytoplasm (Walton, S. T., J. Lab. & Clin. Med., 1938–39, **24**, 1308–1309).

**Goodpasture's Method** as modified by MacCallum for *bacteria* in sections (McClung, p. 152). Fix in Zenker's fluid or in formalin Zenker. Stain thin paraffin sections 10–30 min. in: 30% alc., 100 cc.; basic fuchsin, 0.59 gr.; anilin oil, 1 cc.; phenol crystals, 1 gm. Wash in water. Differentiate in formalin (37% solution of formaldehyde) few seconds until bright red color changes to rose. Wash in water. Counterstain in sat. aq. picric acid 3–5 min. until sections become purplish yellow. Wash again in water. Differentiate in 95% alc. until red reappears and some of it as well as of the yellow is washed out. Wash in water. Stain in Stirling's gentian violet (gentian violet, 5 gms.; 95% alc., 10 cc.; aniline oil, 2 cc.; aq. dest., 88 cc.) 5 min. or more. Wash in water. Gram's iodine solution (iodine, 1 gm.; potassium iodide, 2 gms.; aq. dest., 300 cc.) 1 min. Blot dry. Clear in equal parts aniline oil and xylol until no color is removed. Clear in 2 changes xylol and mount in balsam. Gram-negative bacteria, red; gram-positive ones, blue; tissue red and blue; fibrin deep blue. See his **Polychrome Methylene Blue** and **Carbol-Anilin Fuchsin Methylene Blue.**

**Gordiacea,** see **Parasites.**

**Gordon's Silver Method.** For blood smears, also shows parasites, Gordon, H., J. Lab. & Clin. Med., 1936–37, **22**, 294–298. Dry smears of blood or bone marrow in air and fix in 10% formalin. Wash in water. 2.5% aq. iron alum 10 min. or more. 4 changes aq. dest. Dip in 1% aq. gelatin + 1 or 2 drops 2% sodium carbonate and drain. Wash quickly in aq. dest. Impregnate 5–15 min. in silver solution (Add strong ammonia drop by drop to 5 cc. of 10.2% aq. silver nitrate until ppt. is dissolved. Add 5 cc. 3.1% aq. sodium hydroxide

and redissolve ppt. with strong ammonia. With aq. dest. dilute to 100 cc.). Wash in aq. dest. at 60°C. Reduce in: 10% formalin 90 cc. + 2.5% iron alum 10 cc. Wash in tap water, dehydrate in alcohol, clear in xylol and mount in balsam.

**Gossypimine**, see **Safranin O.**

**Grafts.** Intracoelomic of eye primordium, Joy, E. A., Anat. Rec., 1939, **74**, 461–486. See **Transplantation.**

**Gram's Iodine Solution.** Iodine, 1 gm.; potassium iodide, 2 gm.; aq. dest., 300 cc. A stronger solution may be desirable with only 100 cc. aq. dest.

**Gram's Stains** for bacteria:

1. In smears. *Hucker modification* (McClung, p. 138). Stain 1 min. in equal parts A and B: A = crystal violet (85% dye content, 4 gm.; 95% alc. 20 cc.) B = ammonium oxalate, 0.8 gm.; and aq. dest. 80 cc. After washing in water immerse in: iodine, 1 gm. potassium iodide, 2 gm., aq. dest., 300 cc. 1 min. Then wash in water and dry by blotting. Decolorize 30 sec. in 95% alc. gently moving. Blot and counterstain in: 10 cc. sat. safranin in 95% alc. and aq. dest. 100 cc. Wash and dry. *Kopeloff-Beerman Modification* (McClung, p. 139). Stain 5 min. or more in: 1% aq. gentian or crystal violet, 1.5 cc. mixed before use with 0.4 cc. 5% aq. sodium bicarbonate. Rinse in iodine solution made by dissolving 2 gm. iodine in 10 cc. normal sol. sodium hydroxide and adding 90 cc. aq. dest. and stand 2 min. or more. Blot dry. Add 100% acetone drop by drop with specimen tilted till no more color is removed, less than 10 sec. Dry in air. 0.1% aq. basic fuchsin, 10–30 sec. Wash in water and dry. *Weiss Modification* (Weiss, E., J. Lab. & Clin. Med., 1940–41, **26**, 1518–1519). Make thin, uniform smears and fix over flame. Cover slide with 3% gentian violet in 20% alc., 3–5 min. Wash in warm water. Cover 3–5 min. with iodine, 20 gm.; potassium iodide, 40 gm., aq. dest. 300 cc. Wash with warm water. Decolorize in acetone and wash immediately in water. Counterstain quickly in 2% basic fuchsin in 95% alc. Wash in water, dry and examine.

The use of colloidal iodine has been suggested to improve the reaction between bacteria and stain (Lyons, D. C., J. Lab. & Clin. Med., 1936–37, **22**, 523–524). Methods for preparing colloidal iodine are described by Chandler and Miller (W. L. and E. J., J. Phys. Chem., 1927, **31**, 1091–1096).

2. In sections. *Gram-Weigert method* (McClung, p. 152). Fix in Zenker's fluid. Stain paraffin sections lightly in alum hematoxylin and wash in running water. 1% aq. eosin, 1–5 min., followed by washing in water. Stain ½–1 hr. in anilin methyl violet made by mixing 1 part of A with 9 of B: A. abs. alc. 33 cc.; aniline oil, 9 cc.; methyl violet in excess. B. Saturated aq. methyl violet and wash in water. Lugol's iodine 1–2 min. and wash in water. Blot; dehydrate and clear in equal parts aniline oil and xylol several changes. Wash with xylol and mount in balsam. *Glynn's method.* (Glynn, J. H., Arch. Path., 1935, **20**, 896–899). To make stain triturate 1 gm. crystal violet and 1 gm. phenol crystals in mortar and add 10 cc. absolute alcohol. Before using dilute 10 times with aq. dest., allow to stand 2 days and filter. Stain deparaffinized sections of Zenker (less acetic), Bouin, Helly or 10% formalin fixed material for 2 min. Drain off but do not wash. Add Gram's iodine, 1 min. Differentiate in acetone until no more color is given off, 10–15 sec. Wash in aq. dest. Counterstain in 0.05% basic fuchsin in N/500 hydrochloric acid (see **Normal Solutions**). Drain, do not wash, apply 1% aq. trinitrophenol, ½–1 min. Wash in aq. dest. Dehydrate and differentiate in acetone 10–15 sec., clear in xylol and mount in balsam. Gram + bacteria, violet; Gram−, red; nuclei, light red; cytoplasm, yellow.

**Graven's Gold Chloride** method for nerve endings in muscle (Garven, H. S. D., Brain, 1925, **48**, 380–441). This is Fischer's modification of Ranvier's technique as used in Golgi's Laboratory. Immerse small pieces of tissue in 25% aq. pure formic acid and tease a little to assure penetration 10–15 min. Blot with clean cloth. Place in 1% aq. gold chloride just sufficient to completely cover tissue and shake. Avoid all iron instruments. Cover dish with blue or yellow glass. Leave 20 min. Blot with clean cloth and repeat above treatment with formic acid and gold leaving this time in latter 24 hrs. in absolute darkness. Repeat still again. Pass to glycerin and leave in closed vessel in ordinary light. The sharpness of the intensely purple black nerves in a lightly colored background increases with time. Small pieces can then be transferred to aq. dest. and the individual fibers separated. This is facilitated by dissociation in dilute nitric acid. Wash and make final mounts in glycerin. The author used panniculus carnosus of hedgehog, striated muscle of frog and lizard, extrinsic eye muscle of rabbit and human pectoral muscle.

**Gray, R, B, BB,** see **Nigrosin,** water soluble.

**Green PL**, see **Naphthol Green B.**

**Grenacher**, see **Alum Carmine, Borax Carmine.**

**Grieves'** method for undecalcified dental tissues and bone as outlined by Shipley (McClung, p. 345) is: Fix small pieces in 10% formalin 24–36 hrs. or any other desired fixative. Wash in running water 24 hrs. Then pass through 2 changes of aq. dest. 1 hr. each. Dehydrate through ascending alcohols beginning with 50% alc. Equal parts abs. alc. and chloroform, 2 hrs. Chloroform, 2 hrs. 5% sol. of rosin in chloroform, 2 hrs. 10% sol. rosin, 2 hrs. Sat. sol. rosin until it becomes transparent. Imbed in melted rosin using one after another the rosins in 3 small glass dishes on a heated copper bar, 1 min. each. The chloroform carried over evaporates. The rosin containing the tissue is allowed to cool. The block is ground very thin by hand on a carboriundum stone and polished on a fine hone all grinding being done under luke warm water. The smooth surface is then mounted on a slide with a little melted rosin after which the surface is ground and polished in the same way and the section is ready for mounting or for staining.

**Gross Specimens**, see **Color Preservation.**

**Ground Substance** (intercellular), see **Tissue Fluid.**

**Growth.** Many techniques are now available for the measurement of growth of tissues. Increase in number of cells can be revealed by mitotic counts (**Mitosis**). The amount of bone or of dentine laid down while **Alizarin S** or Madder is in the circulation can be estimated. The amount of radioactive isotropes accumulated is a third method (see **Radioactive Phosphorus**) if the amount increases per unit of time while elimination of the nonradioactive element in question remains the same. Valuable histochemical methods are given by Lowry, O. H. and Hastings, A. B., in Cowdry's Problems of Ageing, Baltimore: Williams and Wilkins, 1942, 936 pp.

**Guanin** appears as white granules in retinal tapetum of certain animals including nocturnal ones. Decreases in amount in regions containing more fuscin. For details see Arey, L. B. in Cowdry's Special Cytology, 1932, **3**, 1218.

**Guarnieri Bodies**, cytoplasmic inclusions in smallpox and vaccinia. See **Inclusion Bodies** and Cowdry, E. V., J. Exper. Med., 1922, **36**, 667–684 for supravital staining with brilliant cresyl blue. For sections **Giemsa's** stain is excellent.

**Hairs.** The hair shaft (above the surface of the skin), the hair root (below it) and the hair follicle (encasing the root) call for somewhat different techniques. Individual cells of the shaft can be isolated by maceration in 40% aq. potassium hydrate, or the shaft and root can be cleared and mounted in balsam for repeated study. In case it is too highly pigmented to permit a clear view of its structure first bleach with hydrogen peroxide. The root and the follicle are to be seen in most sections of hairy **Skin** and require no special technique unless one wishes to study the follicles attached to whole mounts of **Epidermis** or to mark them in order to follow their cyclic changes. For further details see Trotter, M., chapter on Hair in Cowdry's Special Cytology, 1932, **1**, 40–65. Hair containing *Tricophyton* or *Microsporon* fluoresces bright green. See Kinnear, J., Brit. Med. J. 1931, **1**, 791–393 on diagnosis of ringworm.

**Hanging Drop** preparations are mostly employed in the examination of living bacteria and protozoa. A drop of the fluid is simply attached to the under surface of a cover glass which is mounted over a depression in a slide. Equally satisfactory results can usually be obtained by simply mounting under a cover glass on an ordinary slide unless the greater depth of the hanging drop is required. When in **Microdissection** it is necessary to get at the cells from the under surface of the cover glass special chambers and hanging drops are employed.

**Harderian Glands**, fluorescence in mice (Strong, L. C. and Figge, F. H. J., Science, 1941, **93**, 331). Technique for rat is given by Grafflin, A. L., Am. J. Anat., 1942, **71**, 43–64.

**Harris Alum Hematoxylin.** Dissolve 1 gm. hematoxylin in 10 cc. absolute alcohol and 20 gms. ammonium or potassium alum in 200 cc. aq. dest. the latter with the aid of heat. Mix the 2 solutions, bring quickly to boiling and add 0.5 gm. mercuric oxide. Solution turns purple. Cool quickly in cold water bath. Mallory (p. 72) recommends adding 5% of acetic acid.

**Heart**, see **Coronary Arteries, Myocardium, Pericardium, Purkinje Cells** and **Fibers.**

**Heidenhain's Azan Stain** (Heidenhain, M., Ztschr. f. wiss. Mikr., 1915, **32**, 361–372). The following details are from Lee (1928, p. 279): Color sections 1 hr. at 55°C. in 2% aq. azocarmine plus 10 drops glacial acetic acid in small staining jar. Wash in water. Differentiate in 96% alc. 100 cc. plus anilin oil 0.1 cc. until cytoplasm becomes pale pink and nuclei clear red. To hurry differentiation add

2 drops anilin oil. Rinse in 96% alc. containing few drops acetic. Put in 5% aq. phosphotungstic acid about 2 hrs. until connective tissue is completely decolorized. Wash rapidly in water. Stain ½–3 hrs. in following solution diluted with equal or double parts aq. dest.: anilin blue (water sol. Grübler) 0.5 gm.; orange G, 2 gm.; acetic acid, 8 cc.; aq. dest. 100 cc. Examine staining under microscope. Wash in water, dehydrate in abs. alc., clear in xylol and mount in balsam. This is a very useful stain. See also McGregor, L., Am. J. Path., 1929, **5**, 545–557 for use of this technique particularly as applied to normal renal glomerules. Under **Islets of Langerhans** is given use of a slightly modified azan method by Gömöri.

**Helianthin,** see **Methyl Orange.**

**Heliotrope B,** see **Amethyst Violet.**

**Helly's Fluid** is Zenker's fluid in which 5% formalin is substituted for 5% acetic acid.

**Hemalum** (Mayer's) Hematin, 1 gm.; 90% alc., 50 cc.; aq. dest., 1000 cc.; ammonia alum, 50 gms.; thymol, 1 crystal. Keeps better after adding 20 cc. glacial acetic acid and making **Acid Hemalum.** A good nuclear stain when diluted with aq. dest. 1:20. The above formula has been modified by Lillie (R. D., Stain Techn., 1942, **17**, 89–90): hematoxylin, 5 gm.; sodium iodate ($NaIO_3$), 1 gm.; ammonia alum ($AlNH_4(SO_4)_2$ + 12 $H_2O$), 50 gm.; aq. dest., 700 cc., glycerol, 300 cc., glacial acetic acid, 20 cc. No ripening is necessary. Stain sections formalin fixed material, 2–5 min. Blue 2–10 min. in tap water. Counterstain in 0.2% aq. eosin Y. Dehydrate clear and mount as usual. This method is quick and gives a sharp stain.

**Hematin,** identified by luminescence with **Luminol.** Do not confuse with hematein, see **Hematoxylin.**

**Hematoidin** (hematin + G. *eidos*, appearance). An iron free pigment produced by phagocytic digestion of erythrocytes or in clots and old hemorrhages, chemical composition similar or identical with **Bilirubin.** Seen as red or orange rhombic plates or radiating yellow needles, insoluble in ether, water and soluble only with difficulty in alcohol, easily soluble in chloroform. Gives positive **Gmelin's** test.

**Hematoporphyrin** (G. *haima*, blood + *porphyra*, purple). Results from decomposition of hemoglobin. It is hematin minus iron. May appear normally in urine and be greatly increased in amount in certain diseases. Mallory (p. 140) states that it can be decomposed by conc. nitric or sulphuric acid with same green, red, blue color reaction as bile pigment but is insoluble in dilute alkalies and acids and does not bleach.

**Hematoxylin** is the most useful of all dyes in animal histology and pathology (Gr. haimatōdēc, blood like + Xylon, wood). It is an extract of logwood (*Haematoxylon campechianum*) and is marketed in crystalline form. When the crystals are first dissolved in water or alcohol it is not an energetic stain; but requires to be "ripened" before it can be used to advantage. Ripening is brought about by the formation of oxidation products. Consequently it is recommended that solutions be exposed to light and air. Hematein (not hematin—a blood pigment) is the oxidation product which yields a fine deep blue coloration and is the one most desired. It can be purchased. To make up solutions of hematein instead of hematoxylin is logically sound but there is no way to prevent further ripening (oxidation) with the development of other browner unwanted products and precipitation of dyes. Therefore it is good practice to begin with hematoxylin, to let it ripen naturally over a fairly long period of time or to ripen almost immediately by adding about 5% hydrogen peroxide, or 5% of 1% aq. potassium permanganate. 10% solution of hematoxylin in 96% or abs. ethyl alcohol should always be kept on hand. It attains maximum ripening in about one year, but must be kept in a stoppered bottle for otherwise the alcohol will evaporate. It is diluted to 0.5% of hematoxylin with aq. dest. for the **Iron Hematoxylin** technique. See also **Delafield's, Ehrlich's, Harris'** and **Mayer's** hematoxylin solutions, likewise **Azure II eosin** and **Hematoxylin.**

**Hematoxylin and Eosin** is rightly the most used of all staining methods. If the tissues have been fixed in a fluid containing mercuric chloride such as Zenker's fluid deparaffinize sections and treat with dilute iodine in 70% alcohol for 1–2 min. Wash in aq. dest., bleach in 5–10% aq. sodium hyposulphite to remove iodine and wash again in aq. dest. Stain with **Harris' Hematoxylin** (full strength) for 12–15 min. Blue in tap water or in aq. dest. + few drops sat. aq. lithium carbonate, 5–10 min. Stain in 0.2% aq. eosin, 1 min. Rinse in aq. dest. and 95% alcohol. Dehydrate in absolute alcohol, clear in xylol and mount in balsam. Nuclei, deep blue; cytoplasm, pink. In place of Harris' alum hematoxylin, which we use, **Delafield's Alum Hematoxylin** or **Ehrlich's Acid Hematoxylin** may be employed. The Bensleys (p. 73) dilute

1 part of the last named with 2 parts cold sat. aq. ammonium alum and 4 parts aq. dest. Nuclei, dark blue; cytoplasm, collagenic fibers, erythrocytes, pink; smooth muscle, lavender. 0.2% aq. erythrosin can take the place of the eosin but the advantage is questionable.

**Hemin Crystal Test** for blood pigment, Teichmann (Stitt, p. 698). Dissolve in 100 cc. glacial acetic acid, 0.1 gm. of KI, of K Br and of K Cl. Add few drops to suspected material on a slide and cover. Gently warm until bubbles begin, then slowly cool and examine for typical dark brown crystals. The test is not very sensitive but positive result is conclusive.

**Hemochromogen Crystal Test.** Donogány (Stitt, p. 698). Mix 1 drop of suspected fluid, of pyridin and of 20% aq. NAOH on a slide and allow to dry. Radiating crystals appearing within several hours indicate presence of hemochromogen.

**Hemocytoblasts,** see **Erythrocytes,** developmental series.

**Hemofuscin.** Mallory's fuchsin stain. Fix in Zenker's fluid, alcohol or 10% formalin. Stain nuclei in paraffin or celloidin sections with **Iron Hematoxylin.** Wash thoroughly in water. Stain 5–20 min. in: basic fuchsin 0.5 gm., 95% alc. 50 cc. and aq. dest. 50 cc. Wash in water. Differentiate in 95% alcohol, dehydrate in abs. alc., clear in xylol and mount in balsam in the case of paraffin sections. Celloidin sections are to be cleared in terpineol or origanum oil after 95% alc. Nuclei blue, hemofuscin granules bright red, hemosiderin and melanin unstained (Mallory, p. 136).

**Hemoglobin,** histochemical test (Ralph, P. H., Stain Techn., 1941, **16,** 105–106). Flood dried blood smear with 1% benzidine in absolute methyl alc., 1 min. Pour off and replace with 25% superoxol in 70% ethyl alc., 90 sec. Wash in aq. dest., 15 sec. Dry and mount in neutral balsam. Hemoglobin dark brown.

**Hemoglobin Estimation** is done by comparing blood with a colored paper scale or by a more accurate scale in a hemoglobinometer. The experimental error is at least 5%. *Staining reactions* for hemoglobin within cytoplasm (Kindred, J. E., Stain Techn., 1935, **10,** 7–20).

**Hemosiderin,** soluble in acids and other reagents used in histological technique. After formalin fixation the order of decreasing removal is oxalic, sulphuric, nitric, formic and hydrochloric. Speed of solution is but little influenced by age of pigment (Lillie, R. D., Am. J. Path., 1939, **15,** 225–239). See **Iron,** Dinitrosoresorcinol method.

**Heparin.** A method for the histological demonstration of heparin has been described by Jorpes, E., Holmgren, H. and Wilander, O., Ztsch. f. mikr. anat. Forsch., 1937, **42,** 279–301. It is based on evidence that **Tissue Basophiles** contain this substance. See also **Anticoagulants.**

**Heptaldehyde.** An agent said by Strong, L. C., Am. J. Cancer, 1939, **35,** 401–407, to produce liquefaction of spontaneous mammary tumors of mice. It was not helpful when injected into rat lepromata (Cowdry, E. V. and Ruangsiri, C., Arch. Path., 1941, **32,** 632–640).

**Hermann's Fluid.** 2% osmic acid, 4 cc.; 1% platinum chloride, 15 cc.; glacial acetic acid, 1 cc. This resembles Flemming's fluid and is a good cytological fixative.

**Herring Bodies,** see Cushing, H., Proc. Soc. Exp. Biol. & Med., 1932–33, **30,** 1424–1425.

**Herxheimer's** solution for staining fat: scarlet red (scharlach R, sudan IV), 1 gm.; 70% alcohol, 50 cc.; acetone C.P., 50 cc. See **Sudan IV.**

**Heterophile,** see **Staining.**

**Hexuronic Acid** as antiscorbutic factor (Harris, L. J., and Ray, S. N., Biochem. J. 1933, **27,** 58–589).

**Hickson Purple,** a disazo dye, giving in aq. sol. a purple color to leucocytes and a red color to erythrocytes introduced by H. G. Cannan (J. Roy. Micr. Soc., 1941, **61,** 88–94).

**Higgins' Ink.** This was apparently first used as a vital stain by George Wislocki, see Foot (McClung, p. 114). Dilute with equal volume sterile aq. dest. Warm and inject into marginal vein of rabbit's ear 5 cc. daily for 3–4 days, then every 3 days as long as desired. Since the carbon is relatively insoluble it is a simple matter to fix, imbed, section and counterstain. Smaller amounts are to be used for smaller animals, see **Vital Stains.**

**Hirudinea,** see **Parasites.**

**Hischler's Fluid,** see **Golgi Apparatus.**

**Historadiography** is the x-ray photography of tissues. By a special technique Larmaque, P., Bull. d'Hist. Appl., 1937, **14,** 1–16) rays emitted at a tension of 50–100 KV having a length of 0,12–0,2 A° are directed upon a section closely applied to a particularly finely grained emulsion. The absorption of the rays by the section depends upon the density of its parts. Total opacity of the tissue to the rays is marked on the photographic negative by white, permeability by black, and there are usually all grades between the two. Subsequent magnification of about 500 times is possible, but is not advisable. Sections, not more

than 4 microns thick, of formalin fixed tissues, are recommended. An illustrated description of the appearance of epidermis, cartilage, artery wall, thyroid and other tissues is provided by Turchini (J. Bull. d'Hist. Appl., 1937, **14**, 17–28). Historadiography may have many uses in the measurement of densities in different physiological states and in study of the distribution of substances opaque to x-rays experimentally introduced. In some cases great density may accompany high **Viscosity.**

**Histospectrography.** This is a very valuable survey method for minerals in tissues. See Policard, A., Protoplasma, 1933, **19**, 602–629; Scott, G. H. and Williams, P. S., Anat. Rec., 1935, **64**, 107–127; Cowdry, E. V., Heimburger, L. F., and Williams, P. S., Am. J. Path., 1936, **12**, 13–29. Optic lens and cataracts have been analysed particularly for iron, copper and zinc (Busnel, R. G., Pillet, P. and Tillie, H., Bull. d'Hist. Appl., 1938, **15**, 99–109). MacCardle, R. C., Engman, M. F., Jr. & Sr., Arch. Dermat. and Syph., 1941, **44**, 429–440 have employed histospectrography to advantage in determination of skin magnesium. See **Absorption Spectra.**

**Hofmann's Violet** (CI, 679)—dahlia, iodine violet, primula R water soluble, red violet, violet R, RR or 4RN—Conn (p. 120) says above names are applied rather indiscriminately to stains varying in shade from methyl violet to basic fuchsin which are mixtures of methylated and ethylated rosanilins and pararosanilins having less than 5 methyl or ethyl groups. He further remarks that a mixture of basic fuchsin and methyl violet of the color desired may perhaps be made by the worker himself as a substitute for Hofmann's violet which is in fact the composition of some samples sold as Dahlia and Hofmann's violet.

**Huber's Toluidin Blue** stain for Nissl bodies (Addison in McClung, p. 150). This much used method is suggested for autopsy material. Fix in 95% alcohol, 100 cc.; trichloracetic acid (Mallinckrodt), 1.5 gm.; mercuric chloride (Mallinckrodt), 3 gm. 2–10 days depending upon size of piece of tissue. Change fixative every 2 days for larger specimens. Pour off fluid and store in 95% alcohol until used. Do not take out mercury with iodine. Stain paraffin sections in toluidin blue 15–18 hrs. (Make up solution by adding 1 gm. to 500 cc. aq. dest. Heat gently and when it is dissolved add 500 cc. aq. dest.). Pour off stain. Wash in aq. dest. Leave 2 hrs. in lithium carbonate. (Make this by adding 5 gm. to 1000 cc. aq. dest. Boil several minutes. Cool. Filter. To 100 cc. filtrate add 900 cc. aq. dest.). Differentiate in 70% alcohol 5–30 min. Leave flat in 95% alcohol, 5–15 min. Dehydrate in absolute, clear in xylol and mount in balsam.

**Humus,** see soil.

**Hyalin.** This is usually easily recognizable in sections stained with **Hematoxylin and Eosin** or by **Phloxin and Methylene Blue,** by its affinity for eosin or phloxin. **Phosphotungstic Acid Hematoxylin** colors it deep blue. A *hematoxylin-phloxin* method is also recommended by Mallory (p. 207). Fix in alcohol or 10% formalin and imbed in paraffin or celloidin. Stain in alum hematoxylin, 1–5 min. or more. Wash in tap water and stain with 0.5% phloxin in 20% alcohol, 10–30 min. or longer. Wash in tap water and treat for ½–1 min. with 0.1% aq. lithium carbonate. Wash in tap water, dehydrate, clear and mount. In case of celloidin sections, clear in terpineol or origanum oil from 95% alc. Nuclei, blue; fresh hyalin, intensely red; older hyalin, pink to colorless. A simple *thionin* stain is also given by Mallory. It is to stain similar sections for 5–10 min. in 0.5% thionin in 20% alc. Differentiate and dehydrate in 80% alcohol. Then 95% alcohol, terpineol and terpineol balsam. Nuclei and old hyalin, blue.

**Hydrax** is a synthetic resin used as a mounting medium (Hanna, D., J. Roy. Micr. Soc., 1930, **50**, 424–426).

**Hydrogen Acceptors.** These are substances like *p*-amidophenol, *p*-phenylenediamine and resorcin, recommended to strengthen supravital staining of nerve fibers with methylene blue, see **Auerbach's Plexus.**

**Hydrogen Ion Indicators.** Data contributed by Mr. Lester F. Wicks of The Barnard Free Skin and Cancer Hospital.

In 1893 Ehrlich injected neutral red in an attempt to determine the reaction about phagocytosed granules. Since then, other workers have applied other dyes, striving to estimate the approximate pH of tissues, of the fluids bathing them, and even of individual cells. Alizarin red and litmus have been much used, the later especially with lower organisms. Thus, Steiglitz applied all three dyes mentioned above to estimate the reaction of living kidney (E. J., Arch. Int. Med., 1924, **33**, 483–496) and confirmed the contention that alkaline urine can be formed by an acidic cortex. Harvey and Bensley (B. C. H. and R. R., Biol. Bull., 1912, **23**, 225–249) used pH indicators to indicate that gastric fluid does not arise directly within the cells of the mucosa. Margaria (R., J.

Physiol., 1934, 82, 496–497) injected bromcresol purple and bromphenol blue, and claimed to have measured pH changes upon stretching a muscle. Orr (J. W., J. Path. & Bact., 1937, **44**, 19–27) employed phenol red to estimate alterations in pH in the skin of tarred mice during carcinogenesis. Chambers and his colleagues have added pH indicators to tissue cultures (R., Proc. Roy. Soc., B, 1932, **110**, 120–124) and have injected them directly into individual living cells (McClung, pp. 62–109). The most enthusiastic investigator to employ the phthalein and sulphonphthalein indicators is Rous (P., Science, 1924, **60**, 363: J.A.M.A., 1925, **85**, 33–35, and many articles in J. Exp. Med., 1925 to 1927). The literature is extensive but scattered. There are brief reviews by Rous (P., J. Exp. Med., 1925, **41**, 379–411) and von Möllendorf (W., Ergebn. Physiol., 1920, **18**, 141–306).

It is well to question the dependability of data upon pH of living material as apparently indicated by vital staining methods. Consider the ideal requirements for such a vital stain. It should exhibit a sharp and pronounced color change *in the proper pH range*. It should be fairly soluble, readily diffusable, strongly colored, of low toxicity and stable in the organism (not readily oxidized or reduced or precipitated by tissue electrolytes). Of the many indicators employed in analytical chemistry, only a few meet these requirements. Certain errors are to be guarded against in their use. The "salt error" and "protein error" are unavoidably present. In the application of these vital stains changes may take place that will themselves cause a pH change. Among them anesthesia, trauma, loss of carbon dioxide from exposed tissues, interference with blood supply, and postmortem change deserve special mention. However crude though the methods may be, these dye indicators are of value in preliminary experiments or where no better procedure is applicable.

The indicator dyes of most promise are certain of the phthalein and sulphonphthalein compounds. They are generally quite soluble, highly diffusable, show marked color shifts and are fairly constant in composition. The dye solutions diffuse quickly when injected, and quickly appear in the urine and stools. For these reasons, fairly large doses given intraperitoneally are more suitable than subcutaneous injections. But it is doubtful, according to Chambers (personal communication), whether the more soluble dyes actually penetrate the walls of most cells.

The following selection of indicators is based upon the reports of Rous and others, and upon experiments with mice carried out at The Barnard Free Skin and Cancer Hospital. Their chemical names can be found in *The Merck Index* or in any good textbook of chemistry. Some are to be used in 1% aq. solutions, others in sat. solutions in physiological saline, litmus in either aqueous or agar solution (Rous, P., J. Exp. Med., 1925, **41**, 379), while the remainder, which are acidic (the sulphonphthaleins and methyl red), require to be converted to their corresponding sodium salts because the latter are more soluble in water. Consequently the proper equivalent of sodium hydroxide must be reacted with each compound. Rub up 0.1 gm. of the dry dye in a mortar (agate, preferably) with the volume of N/20 sodium hydroxide solution given in cc. below the dye in the table. Filter, wash out the mortar with several small portions of saline (0.9% NaCl) and make all to a volume of 10 cc. For a mouse, 0.5–2.0 cc. of the dye solution should be injected intraperitoneally.

**HYDROGEN ION INDICATORS**

| Indicator | pH Range and Colors | Remarks |
|---|---|---|
| Bromphenol blue 3.0 N/20 NaOH | yellow ← 3.0 — 4.6 → blue | Very strong stain, too far on acid side. |
| Sodium alizarin sulphonate (Alizarin red) 1% aq. or sat. in saline | yellow ← 3.8 — 5.0 → pink | Very toxic, weak stain. |
| Bromcresol green 2.9 N/20 NaOH | yellow ← 4.0 — 5.6 → blue | Strong stain, persistent, well tolerated. |
| Methyl red 7.4 N/20 NaOH | red ← 4.2 — 6.3 → yellow | Unstable in organism, weak stain, fixes on lipoids. |
| Chlorphenol red 4.7 N/20 NaOH | yellow ← 4.8 — red — 6.8 → purple | Powerful stain, well tolerated. |
| Bromcresol purple 3.7 N/20 NaOH | yellow ← 5.4 — 6.6 → purple | Strong stain but rapidly excreted, is toxic and exhibits dichromaticism. |

**HYDROGEN ION INDICATORS**—*Continued*

| Indicator | pH Range and Colors | Remarks |
|---|---|---|
| Bromphenol red 3.9 N/20 NaOH | yellow ← 5.4 — red — 7.0 → purple | Very strong stain, well tolerated. |
| Methyl violet 1% aq. or sat. in saline | blue-violet ← 6.0 — 7.0 → violet | Weak stain, toxic. |
| Bromthymol blue 3.2 N/20 NaOH | yellow ← 6.0 — 7.4 → blue | Weak stain, very toxic to mice, but not for insects. |
| Phenol red 5.7 N/20 NaOH | yellow ← 6.6 — 7.8 → red (6.8 — 8.4) | Rapid, intense stain, very well tolerated. |
| Litmus, purified (Azolitmin) 1% aq. or in agar sol. | (approx.) red ← 6.0 — 8.0 → blue | Slow stain, diffuses poorly, usually deposits in granules. |
| Neutral red (Toluylene red) 1–2% aq. or sat. in saline | (approx.) red ← 6.8 — 8.0 → yellow | Very weak stain, precipitates out readily *in vivo*, not toxic if pure. |
| Cresol red 5.3 N/20 NaOH | yellow ← 7.2 — 8.4 → purple-red | Somewhat toxic, not a strong stain. |
| Metacresol purple 5.3 N/20 NaOH | yellow ← 7.4 — 9.0 → purple | Very weak stain, not very soluble. |
| Thymol blue 4.3 N/20 NaOH | yellow ← 8.2 — 9.4 → blue | Toxic, range too alkaline. |

**Hydrokollag,** a particulate material employed for injection of **Lymphatic Vessels** which see.

**Hydrotropes,** see **Sudan Stains.**

**Hydroxy Tri-Phenyl Methanes.** These are the rosolic acids. Amino groups of triamino tri-phenyl methanes are replaced by hydroxyls making them acidic instead of basic. Examples: aurin (or rosolic acid); red corallin.

**Hydroxyquinoline** test for iron, see **Iron.**

**Hypophysis,** see **Pituitary.**

**Idiochromatin** (G. idiōs, one's own, peculiar). The chromatin concerned particularly with reproductive functions such as chromosome formation contrasted with nutritive trophochromatin (G. trophē, food, nourishment). There is no special technique for it.

**Illumination.** For microscopic work the lighting is of great importance. Direct visible light can best be obtained from various electric microscopic lamps on the market. Only when the light is more intense than that required for routine purposes can it be properly employed for dark field examination or for polarization. Therefore an intense source should be available. The intensity can be reduced to optimum by using an iris diaphragm. When it is desired to deliver light into the body to a position behind living tissues or organs for transillumination the **Quartz Rod** technique is suggested.

Even to make the light equivalent in quality to that from the white cloud on a bright day, that microscopists used to search for, is quite unnecessary. If the light is too much screened by "daylight" or other glass its intensity will be impaired. *Green light* was recommended quite enthusiastically about 20 years ago. But it is difficult to secure green light of the necessary intensity and it is unpleasant to work with. Ultraviolet light, which permits higher resolution and is selectively absorbed especially by nucleoproteins, is used occasionally for **Ultraviolet Photomicrography.** The objects, however, can of course not be seen directly so that to photograph them is a hit and often miss experience, though it is possible to focus on a fluorescent screen. The principal use of ultraviolet light is in the **Fluorescence Microscope** by which the structures giving off fluorescence can be viewed in a dark background at high magnification.

**Imbedding,** see **Celloidin, Paraffin, Glycol-Stearate, Rubber Paraffin, Ceresin, Double** and **Gelatin** for imbedding preparatory to sectioning. The **Mounting** of sections and whole tissues is a kind of imbedding.

**Immunization** of monocytes against foreign erythrocytes with phagocytosis of the latter (Bloom, W., Arch. Path. and Lab. Med., 1927, **3,** 608–628).

**Impedence,** see **Electrical Resistance.**

**Imperial Red,** see **Eosin B** or bluish.

**Imperial Yellow,** see **Aurantia.**

**Impression Preparations,** see **Smears.**

**Inanition,** see **Fasting.**

**Inclusion Bodies** are any substances included in a cell, tissue or organ. There is the implication that the substance is included from without, that is to say, it is of extraneous origin. But the designation is so loosely used as to be almost meaningless. It is applied to droplets of fat, ingested pigments, remnants of phagocytosed materials, bodies developed in cells as a result of virus action and so forth. The virologists have taken over the designation from normal cytology in which it is used less and less. In certain virus diseases inclusions form in the nucleus, in the cytoplasm or in both (Cowdry, E. V. in Rivers' book on Virus Diseases, Baltimore, Williams & Wilkins, 1928, pp. 113–154).

Since the nucleus is shielded from the environment by the cytoplasm its reactivity is restricted and the materials available for the formation of nuclear inclusions are also limited as compared with those in the cytoplasm. Consequently the composition of nuclear inclusions in virus diseases is more uniform than that of cytoplasmic inclusions. See **Nuclear** and **Cytoplasmic Inclusions**

**Indamin Dyes.** Methylated amino derivatives of indamin. Bindschedler's green and toluylene blue.

**India Ink,** see **Higgins'.**

**Indicators,** see **Hydrogen Ion** and **Oxidation Reduction Potential** indicators.

**Indigo** (CI, 1177). Produced both from plants and artificially.

**Indigo-Carmine** (CI, 1180)—indigotine Ia—This sodium salt of indigosulfonic acid is blue with acid characteristics so that it is a good counterstain for carmine. It has been employed with fuchsin by Shumway, W., Stain Techn., 1926, **1,** 37–38. See renal excretion of (Kempton, R. T., Bott, P. A. and Richards, A. N., Am. J. Anat., 1937, **61,** 505–521). It was used as a vital stain by Heidenhain who employed 35–60 cc. of 0.4% suspension for rabbits and 150–1500 cc. for dogs (see Foot, McClung, p. 113). The Bensleys (p. 151) advise intravenous injection of 4 cc. sat. filtered aq. indigo-carmine per kilogram of body weight. Fix by vascular perfusion with formalin alcohol (neutral formalin, 10 cc.; absolute alcohol, 90 cc.) or by immersion in it. Counterstain frozen sections with **Mayer's Acid Carmine** or with 1% acridine red. Another way is to imbed (in paraffin), section, clear and examine with or without this counterstaining.

**Indigotine Ia,** see **Indigo-Carmine.**

**Indin Blue 2rd,** see **Naphthol Blue R.**

**Indo Reaction** for phenols. Formation by oxidation of an aromatic paradiamine in presence of tissue phenol of a blue or green indamine. A difficult reaction (Lison, p. 142). See Lison's study of the venom gland of toads (Lison, L., C. Rend. Soc. de Biol., 1932, **111,** 657–658).

**Indol Compounds,** see **Nitro Reaction, Nitrosamino Reaction.**

**Indophenol Blue** (CI, 821). This is formed by oxidation of a mixture *p*-aminodimethylaniline and $\alpha$ naphthol. Conn (p. 73) says that this is probably the dye employed for staining fat by Herxheimer, G., Deut. Med. Wochenschr., 1901, **27,** 607–609.

**Indophenol Oxidase,** see **Nadi Reagent, Cytochrome, Oxidase.**

**Indophenols.** Dyes closely related to indamines. Example: indophenol blue.

**Indulin.** 1. Spirit soluble (CI,860)—spirit indulin and spirit nigrosin R.

2. Water soluble (CI, 861)—fast blue B, OB, R, etc., soluble indulin 3B—An infrequently used acid azin dye. Lynch, J. E., Zeit. f. wis. mikr., 1930, **46,** 465–469; Cumley, R. W., Stain Techn., 1935, **10,** 53–56.

**Indulin Black,** see **Nigrosin,** water soluble.

**Infra Red** photography shows split appearance of chromosomes (Ganesan, D., J. Roy. Micr. Soc., 1939, **59,** 75–78) and gives better definition of epiphyseal layers of normal and rachitic bone (Siegel, L., Allen, R. M., McGuire, G. and Falk, K. G., Am. J. Path., 1939, **15,** 273–277). Guardabassi, M., C. rend. Soc. de Biol., 1935, **118,** 559–561 has used this technique for alcohol fixed sections of brain of rabid dog sensitized with rubrocyanine to demonstrate structure of Negri bodies. Transmission of infra red light through the skin facilitates photography of superficial veins in the living state. Resolution with this light of relatively long wave length is inferior to that with visible light.

**Injection,** see **Microinjection. Perfusion** of blood vessels and **Neutral Red** method of staining pancreas by vascular injection.

**Innervation,** determination by dissection (Wharton, L. R., Anat. Rec., 1937, **67,** 467–475). Place tissue sheets or thin organs on writing paper. Allow to adhere 5–10 min. Place in 1 part glycerol, 1 part glacial acetic acid and 6 parts 1% aq. chloroal hydrate, 18 hrs. Glycerol, 1 part; Ehrlich's hematoxylin, 1 part; and 1% aq. chloral hydrate, 6 parts, 24 hrs. or more. If overstained decolorize in first solution or in 1% hydrochloric acid in 70% alcohol. Transfer to glycerol 10 days. Dissect under binocular microscope in fresh

glycerol. To make permanent preparations, pass up to 95% alcohol, then through bergamot oil, 2 parts; cedar oil, 1 part; and pure carbolic acid liquefied by heat, 1 part, to xylol. Mount in balsam. See **Nerve Endings.**

**Insects.** For whole mounts of *large insects* Stapp, P. and Cumley, R. W., Stain Techn., 1936, **11**, 105–106, specify abs. alc., 5–15 days; 95, 85, 70, and 50% each 15 min. Alc. 35%, 30 min. Equal parts $H_2O$ and $H_2O_2$ + trace $NH_4OH$, 12–24 hrs. Alc. 35, 50, 85, and 95%, 15 min. each. Abs. alc. 2–3 changes, 3 days or more. Toluol, 10–21 days. Pass from thin to thick dammar and mount. Perhaps the simplest method for *small insects* (fleas, etc.) is simply to drop them in creosote, U.S.P. and after 24 hrs. to mount them directly in balsam (Fox, I., Science, 1942, **96**, 478). Sectioning is facilitated by methods designed to soften **Chitin,** see also **Fleas, Ticks.**

**Intestine.** Difference in appearance of wall when contracted and normally distended (Johnson, F. P., Am. J. Anat., 1912–13, **14**, 235–250). Alterations in human mucosa from absorption of fat and from fasting (Cowdry's Histology, pp. 302–305). Effect of different dehydration and clearing agents on intestine (Ralph, P., Stain Techn., 1938, **13**, 9–15). Rosenberg, L. E., Stain Techn., 1940, **15**, 53–56 has given an interesting account of postmortem autodigestion. Mingazzini phenomenon (Macklin, C. C. and M. T., J. Anat., 1926, **61**, 144–150). See **Large** and **Small Intestines.**

**Intracellular Phase,** see **Chloride.**

**Intranuclear** crystals. Hepatic cells of dogs. Determination of properties (Weatherford, H. L., and Trimble, H. C., Anat. Rec., 1940, **77**, 487–502).

**Iodeosin B,** see **Erythrosin,** bluish.

**Iodine,** detection of: 1. Ionized iodine in the form of iodides. Stieglitz (E., J. Pharm. and Exp. Therap., 1924, **22**, 89–98) injects 20 cc. 5% aq. lead nitrate intravenously into an animal to be killed and fixes the tissue in formalin. In the sections, iodine is found in the form of yellow crystals of lead iodide. Methods have been reviewed by Gersh and Stieglitz (I. and E. J., Anat. Rec., 1933, **56**, 185–193).

2. Methods for iodine in organic combination appear to be unsatisfactory. The whole subject of iodine has been critically considered by Lison (p. 111–113). See **Gram's** and **Lugol's** solutions.

**Iodine Green** (CI, 686), closely related to methyl green, only used occasionally.

**Iodine-Iodide Solution.** This term is employed for almost any solution containing iodine and iodide as **Lugol's** and **Grams.**

**Iodine Violet,** see **Hofmann's Violet.**

**Iris Blue,** see **Resorcin Blue.**

**Iris Violet,** see **Amethyst Violet.**

**Iron** occurs in tissues "masked" in organic compounds which are not ionisable and free in inorganic compounds which are ionisable into ferric and ferrous salts.

1. *Macallum's hematoxylin* method depends upon the formation of a blue black iron hematoxylinate. The tissue is fixed in 95% alcohol 24–48 hours, dehydrated, cleared, imbedded in paraffin and the sections are passed down to distilled water. Contact with iron is reduced to a minimum. The microtome knife must be free of rust. Treat sections with a freshly prepared straw yellow 0.5% aqueous solution of hematoxylin which must be of the highest purity. *Inorganic iron* produces the blue-black compound which is relatively insoluble. Dehydrate, clear and mount in balsam in the usual way.

The technique for *organic iron* is more difficult because it must be unmasked before it will react in this way. The best account is Nicholson, F. M., J. Comp. Neurol., 1923, **36**, 37–87. In studying the cytoplasmic iron containing proteins of nerve cells of the medulla of rats, he fixed in 95% alcohol 48 hours; dehydrated in absolute alcohol 2–5 hours; cleared in cedarwood oil until transparent; imbedded in paraffin (2 changes) and cut sections 7$\mu$. After being deparaffinized, the sections were passed through alcohols to 4% pure sulphuric acid in 95% alcohol held at 60°C. for 5–60 minutes. This liberated the iron. The sections were washed in 95% alcohol; passed down through graded alcohols to aq. dest., and placed in freshly prepared 0.5% aqueous hematoxylin, 1–5 minutes in which the blue-black hematoxylinate forms. Then wash in aq. dest. (not tap water). Counterstain in dilute alcohol erythrosin and mount as usual. As a check the nuclear chromatin of sections not treated with the acid alcohol should not be colored black by this hematoxylin solution. Difficulty may be experienced because the color of the unmasked iron is faint. The reaction is a chemical one of great delicacy and requires practice. Pancreatic acinous cells also afford favorable material. Look for cytoplasmic iron in the poles distant from the lumen where the chromidial material, which resembles the Nissl bodies, is most concentrated.

2. *Prussian blue reaction.* Prepare sections in the same way, deparaffinize and test as described in Lee (p. 291).

For ferric salts of inorganic iron wash in aq. dest., 2% aqueous potassium ferrocyanide, 3–15 minutes; Prussian blue is formed, wash, dehydrate, clear and mount. For ferrous salts substitute ferricyanide for ferrocyanide in the test. For both use equal parts of ferrocyanide and ferricyanide. When the iron is organic it is unmasked by treating the sections with 3% pure nitric acid in 95% alcohol for 24–36 hours at room temperature or at 35°C. if necessary. Wash in pure 90% alcohol and in aq. dest. Place in equal parts freshly made of 1.5% aqueous potassium ferrocyanide and 0.5% aqueous hydrochloric acid for not more than 5 minutes. Wash well in aq. dest., colored with eosin or safranin, dehydrate, clear and mount.

Hemosiderin gives Prussian blue reaction for inorganic iron. The iron in hemoglobin is not unmasked by these acid alcohols. Brown, W. H., J. Exper. Med., 1911, **13,** 477–485, devised special methods for its demonstration. Testing for iron in association with calcium particularly in bone is critically described by Cameron, G. R., J. Path. and Bact., 1930, **33,** 929–955. He emphasizes the fact that exposure of tissues and fluids to dust in a city like London is an important source of error.

3. *Microincineration* yields a mineral residue that contains iron originally both organic and inorganic. Color of the iron oxides, viewed in the dark field, varies according to Policard (C. rend. Acad. d. sc., 1923, **176,** 1187) from yellow to deep red. He suggests that perhaps the yellow to brown ash is of organic iron and the red ash is of free iron. See also Marza, V. D., Marza, E., and Chiosa, L. Bull. d'hist. Appliq., 1932, **9,** 213. Scott (McClung, p. 758) warns against confusion with carbon.

4. *Hydroxyquinoline test* (Thomas, J. A. and Lavollay, J., Bull. d'Hist. Appl., 1935, **12,** 400–402). Fix in alcohol, trichloracetic acid or neutral formalin. Avoid formol with alkaline water and fixatives containing chromium. Make up reagent by dissolving 2.5 gm. 8-hydroxyquinoline in 4 cc. pure acetic acid warming gently. Add quickly aq. dest. to make 100 cc. Filter. Wash sections (or smears or cultures) well in neutral aq. dest. Then add few drops of reagent 5–15 min. Pour off reagent. Add to preparation 1 drop 25% aq. ammonia which produces a ppt. Wash in a stream of neutral aq. dest. If large crystals remain wash more energetically. Stain nuclei with lithium carmine. Examine in neutral aq. dest. or dehydrate in terpinol and mount in vaseline oil. Iron, green black; nuclei, red. Recommended for localization of iron in granules of vitellus, in red blood cells, and in connection with microincineration. Said to be better than **Prussian Blue** reaction for iron.

5. *Dinitrosoresorcinol* (Humphrey, H. A., Arch. Path., 1935, **20,** 256–258). Treat paraffin sections of formalin fixed tissue with 30% aq. ammonium sulphide, 1 min. Rinse in water and immerse in sat. aq. dinitrosoresorcinol (Eastman) 6–20 hrs. A counterstain can be employed. Humphrey does not say which. 1% eosin in 50% alcohol should be satisfactory because the iron containing compounds such as hemosiderin are colored green. Wash, dehydrate, clear and mount.

Intravenous injections of *colloidal* solutions of *iron* in rabbits are described by Duhamel, B. G., C. rend. Soc. de Biol., 1919, **82,** 724–726.

**Iron Hematoxylin** of Heidenhain is one of the standard stains. It will give excellent results after almost any good fixation. Zenker's fluid and formalin-Zenker are suggested. Bring paraffin sections down to aq. dest. Mordant in 5% aq. iron ammonium sulphate (iron alum, light violet colored crystals, discard the brownish material accompanying them) 12–24 hrs. Rinse quickly in aq. dest. Transfer to 1% aq. hematoxylin (made up by diluting 1 cc. sat. sol. hematoxylin in abs. alc. with 99 cc. aq. dest.) for 12–24 hrs. Differentiate under microscope in 1% aq. iron alum. Wash thoroughly in tap water. Many counterstains can then be used such as 1% aq. Bordeaux red, orange G., acid fuchsin, acridine red, or **Mucicarmine.** Dehydrate, clear and mount. Nuclei dense blue-black in background of color selected. See **Centrosomes, Nuclei, Regaud's Method** for mitochondria.

1. Koneff, A. A., Anat. Rec., 1936, **66,** 173–179 advises use with anilin blue. Mordant sections 5–10 min. in 5% aq. iron ammonium sulphate. Rinse quickly in aq. dest., stain 3–15 min. in Harris' hematoxylin. Rinse again in aq. dest. and stain in: anilin blue (Grübler) 0.1 gm.; oxalic acid, 2 gm.; phosphomolybdic acid, 15 gm. and aq. dest. 300 cc. Wash in aq. dest., differentiate in alcohol, dehydrate (2 changes of absolute), clear in xylol and mount in balsam. If euperal is used for mounting omit the xylol. Nuclei, violet-brown; cytoplasm, light brown; erythrocytes, dark violet; myelin and muscle brown; elastic fibers, reddish brown to red.

2. Lillie, R. D. and Earle, W. R., Am. J. Path., 1939, **15,** 765–770 recommend employment of a hematoxylin containing ferric and ferrous iron: (A).

Ferric ammonium sulphate, violet crystals, 15 gm.; ferrous sulphate, 15 gm.; aq. dest., 100 cc. (B). Hematoxylin, 1 gm.; 95% alcohol, 50 cc., glycerin, C.P., 50 cc. Mix A and B in equal quantities before using.

**Iron Pigments,** see **Berlin** and **Turnbull** blue reactions.

**Iron, Radioactive.** See **Erythrocytes.**

**Isamine Blue** is described by Conn (p. 137) as a sulfonated naphthyl-rosanilin or naphthyl-pararosanilin. He questions the synonym (alkali blue XG) given in the Colour Index. This acid has been much used as a **Vital Stain** in European laboratories. It is not made in the United States.

**Islets of Langerhans** of the pancreas. There are many techniques for the study of these cellular masses.

1. To study in the living state the method employed by O'Leary, J. L., Anat. Rec., 1930, **45**, 27–58 is recommended. It consists essentially of partly withdrawing the pancreas from a mouse and of mounting it in such a way that a thin film of tissue can be closely examined with circulation still active. The islet cells can be studied with oil immersion lenses and the changes in them on the injection of insulin noted.

2. To obtain an idea of the distribution, number and size of the islets supravital staining with **Neutral Red** or **Janus Green** is indicated, which see.

3. To stain the cell types specifically **Neutral Gentian** and other stains advised by Lane, Bensley and their followers are available. The **Azan Stain** suggested by Bloom, W., Anat. Rec., 1931, **49**, 363–371 (see his beautifully colored plate), has been further investigated by Gomori, G., Anat. Rec., 1939, **74**, 439–459 whose technique abbreviated is as follows: Fix thin slices of pancreas in Bouin's fluid 8–10 hrs. Wash in aq. dest. Imbed in paraffin and cut 4$\mu$ sections. Stain 45–60 min. at 56°C. in azocarmine. (To make dissolve 0.1% azocarmine in aq. dest. Boil about 5 min. Cool and add 1.0 cc. glacial acetic acid to each 50 cc. solution. Before use filter at 60°C. Stain will keep for months.) Rinse quickly in aq. dest. and blot. Destain in 90% alcohol containing 1% aniline oil until acinous tissue is almost wholly decolorized and B cells show red against pink background of A cells. Rinse briefly and treat with 5% aq. iron alum for 5 min. or more. Rinse again and stain 2–20 min. in the usual mixture (anilin blue, 0.5 gm.; orange G, 2.0 gm.; + aq. dest. to make 100 cc.) diluted with 2–3 times its volume of aq. dest. until under the microscope collagenic tissue becomes deep blue. Rinse and blot. Differentiate and dehydrate in absolute alcohol, clear in xylol and mount in balsam. Cytoplasm of A cells rich orange yellow, of B cells fiery red and of D cells sky blue. The author states that by first staining with Bensley's neutral gentian, decolorizing and restaining by above Azan method it can be seen that there is no gradation between A and B cells.

**Isoelectric Points** of cellular structures. Methods for their determination at controlled pH's by intensity of staining have been critically evaluated by Levine, N. D., Stain Techn., 1940, **15**, 91–112. His conclusion is that no true isoelectric points have yet been established for nucleus, cytoplasm or other tissue elements by these techniques. See reticulo-endothelial cells (Fautrez, J., Bull. d'Hist. Appl., 1936, **13**, 202–206).

**Isohematein,** as a biological stain (Cole, E. C., Stain Techn., 1931, **6**, 93–96). Greater tinctorial power than hematoxylin but less selective.

**Isopropyl Alcohol.** Has been recommended as a substitute for ethyl alcohol since it mixes with water and xylol. It is said to be less hardening than ethyl alcohol (Bradbury, O. C., Science, 1931, **74**, 225) but it is more expensive. See Herman, C. M., J. Lab. & Clin. Med., 1941, **26**, 1788.

**Isorubin,** see **New Fuchsin.**

**Iso-Safrol** is obviously an isomer of safrol which is given as 3,4-methylene-dioxy-allylbenzene in the Merck Index. Isosafrole is listed among Eastman's organic chemicals. It is sometimes recommended as a partly dehydrating and clearing agent (**Silver Citrate** injection of blood vessels, etc.) but in all likelihood other clearing agents can be used as substitutes.

**Jacobson's Organ,** innervation, Bellairs, A., J. Anat., 1942, **76**, 167–177.

**Jalowy** modification of Hortega method for the skin (Jalowy, B., Zeit. f. Zellf. u. Mikr. Anat., 1937, **27**, 667–690). To make reagent wash ppt., formed by adding 20 drops 40% aq. NaOH to 20 cc. 10% aq. silver nitrate, 10 times with aq. dest. Suspend ppt. in 20 cc. aq. dest. Add ammonia drop by drop till it dissolves. Add 100 cc. aq. dest. and store in dark. Deparaffinize sections of tissue fixed 1–2 days in neutral formalin. Treat with above reagent 5–30 min. at 30°C. Rinse in aq. dest. and in ammonia water. After treating with 1 part neutral formalin to 4 of aq. dest. wash in running water, dehydrate, clear and mount in balsam. Collagen, yellow to brownish yellow; reticular fibers, black.

**Janssen's Iron Hematoxylin** recommended in place of Weigert's acid iron chloride, hematoxylin (Lillie, R.D. and Earle W.R. Stain Technol., 1939, **14**, 53–54).

**Janus Blue** can be used in exactly the same ways as Janus green and with equal success.

**Janus Dyes.** Named after the God, *Janus* with two faces since they often exhibit two colors. Their chemistry and use in histology is described by Cowdry, E. V. Contrib. to Embryol., Carnegie Inst. Washington, 1918, No. 25, pp. 39–148.

*Janus green* (formerly made by Grubler) is safraninazodimethylanilinchloride. This is useless for staining mitochondria.

*Janus green C* (Hoechst) is dimethyl safraninazodimethyl anilinchloride. This likewise is useless for mitochondria.

*Janus green B* (Hoechst) is diethylsafraninazodimethylanilinchloride. This is the most specific stain for mitochondria and is now supplied by many companies both as Janus Green B and simply as Janus Green.

*Janus blue G and R* (Hoechst) is diethylsafranin-B-naphthol and stains mitochondria as well as Janus Green B. The marks G and R indicate differences in method of manufacture not different dyes.

*Janus black D, I, II and O* (Hoechst), of these Janus Black I is a mixture of two substances Janus green B and a brown dye. It colors mitochondria by virtue of the former.

*Janus gray B, BB* (Hoechst) are also safranin derivatives but useless for mitochondria.

*Janus yellow G, R,* (Hoechst) likewise safranin derivatives and no good for mitochondria.

*Diethylsafranin* is a reduction product of Janus green B. It is a red dye which colors mitochondria specifically but not very strongly.

**Janus Green B** (Diazingrün) is diethyl safraninazodimethylanilinchloride. Janus green now sold without the qualification B is usually the same substance because it has become well known that the dye required must have the composition indicated. Owing to its toxicity Janus green cannot be injected into living animals like trypan blue and other "vital" stains. It is employed as a supravital stain by simply immersing tissues in it or better by its injection into the vessels of a freshly killed animal the individual cells of which remain for some time alive. Janus green is the best supravital stain for mitochondria. Janus green is also very useful for staining the islets of Langerhans of the pancreas and the renal glomeruli of the kidney when injected intravascularly, see **Neutral Red.** Both islets and glomeruli are colored deep bluish green against a background at first colorless, or faintly green, and changing to pink by reduction of the dye to diethylsafranin. This permits the counting of islets and glomeruli in pieces of tissue mounted in salt solution and observed at low magnification. When the oxygen is further consumed by the cells the dye is reduced to a second colorless leucobase. It is therefore an oxidation-reduction indicator as well as a specific stain for mitochondria. See **Neutral Red-Janus Green** stain.

**Jaws,** see **Teeth** *and*

**Jenner-Giemsa** method of Pappenheim (*see* May-Giemsa).

**Johnson's Neutral Red** stain for Nissl bodies (Addison in McClung, p. 450). Ripen 1% aq. neutral red 1–4 years. Dilute to 0.25–0.5% before using. Differentiate and dehydrate in the usual way. Clear in 1 part xylol + 2–3 parts castor oil. Gives good results in thick sections (50$\mu$) and can be employed after silver methods on tissues fixed in alcohol or formalin.

Kirkman, I. J., Anat. Rec., 1932, **51**, 323–326 used the following unripened stain after Bouin and formalin fixatives: neutral red (Coleman & Bell), 1 gm.; aq. dest., 500 cc., 1% aq. glacial acetic acid, 2 cc. 10–20 min. is sufficient for counterstaining **Weigert-Pal** preparations. Then rinse in aq. dest., differentiate in 95% alcohol, dehydrate in absolute, clear and mount.

**Joints.** *Meniscus* (Raszela, F., Bull. d'Hist. Appl., 1938, **15**, 186–210).

**Jores' Solution,** see under **Color Preservation** of gross specimens.

**Kabunylin,** a dye extracted from beetroot. Said to be good for use with picrofuchsin (Fuse and Hino, Arb. Anat. Inst. zu Sendai, 1937, **20**, 111–113).

**Kaiserling's Solution,** see under **Color Preservation** of gross specimens.

**Kallichrom,** a combination of cresyl violet and auramin recommended for both plant and animal tissues (Kisser, J., Mikr. f. Naturfreunde, 1931, **9**, 95).

**Kardos-Pappenheim** modification of Giemsa's stain (Kardos, E., Folia haematol., Archiv., 1911, **12**, 39). To make the methyl green-orange stain mix 2% aq. orange G. with concentrated aq. methyl green. Filter, dry the ppt. and dissolve in methyl alcohol. Shake well together 5 drops methyl green-orange, 10 drops of Giemsa's stock solution and 15 cc. aq. dest. The fluid under the foam is used for staining. First fix and stain the blood smear with May-Grünwald

mixture 3 minutes; add equal volume aq. dest., 1 minute; pour off and add the methyl green-orange 15 minutes; wash quickly in water and blot dry.

**Karo,** white corn syrup (Corn Products Co.) is a useful medium for mounting whole insects because they can be transferred to it directly from water or weak alcohol and clearing is unnecessary (Patrick, R., Science, 1936, **33,** 85–86).

**Karotin,** see **Carotin.**

**Karyosome** (G. *Karyon* nut, nucleus + *soma,* body). A basic staining or chromatin-nucleus, in contrast to a *plasmosome,* generally more numerous, smaller and of less regular shape often called a net-knot.

**Kerasin** is a **Cerebroside.**

**Keratin,** a scleroprotein contained in hair, nails, horns, epidermis, etc. There are apparently two sorts. Their chemistry is discussed by Giroud, A., Bulliard, H. and Lebond, C. P., Bull. d'Hist. Appl., 1934, **11,** 365–373. See **Orange II, Oral Mucosa.**

**Keratohyalin.** Hyalin-like granules found in the stratum granulosum. They can be beautifully stained with *picrocarmine.*

**Kidney.** Techniques for the sustaining tissues of the kidney (connective tissue, blood vessels, nerves and lymphatics) are essentially the same as those used for the same tissues in other organs. See, however, the **Silver Citrate** injection of blood vessels. The epithelial components are highly specialized and can be investigated in a host of different ways of which only a few samples can be given.

A clear distinction between glomeruli and the remainders of the renal tubules is important. It is a simple matter to color the former with 1:5000 Janus blue (which is more satisfactory for this purpose than Janus green) in 0.85% aq. sodium chloride by vascular **Perfusion** and to determine their number, size and distribution against a background of unstained or faintly rose tinged tubules in slices of fresh kidney (Cowdry, E. V., Contrib. to Embryol. Carnegie Inst., Washington, 1918, **8,** 39–160).

Individual renal tubules in their entirety can be isolated by maceration and teasing as described by Huber, G. C., Cowdry's Special Cytology, 1932, **2,** 935–977 slightly amplified. Partly wash out blood by injecting physiological saline into the renal artery. Then follow with hydrochloric acid (conc. HCl, 3 parts and aq. dest. 1 part) using care to protect the eyes. Remove and immerse the organ in the same fluid. After a suitable time, determined by excising pieces, wash a block of tissue with aq. dest and stain in **Hemalum.** Wash in very dilute aq. sodium hydrate. Isolate individual tubules by teasing with fine needles. Wash, and mount in glycerin. With small mammals Huber's results were excellent but he was not satisfied with his human preparations. The method has however been well adjusted to the human kidney by Oliver, J. and Lund, E. M., J. Exp. Med., 1933, **57,** 435–483 and Arch. Path., 1934, **18,** 755–774. Technique for the microscopic study *in vivo* of the surface of the guinea pig's kidney, for the marking of single tubules with India ink and for their later isolation by maceration is given by Walker, A. M. and Oliver, J., Am. J. Physiol., 1941, **134,** 562–595. The micro collection of fluid from single tubules is as the authors state a direct continuation of the researches of A. N. Richards.

Vital staining of renal tubules is usually carried out by techniques not requiring special adaptation to the kidney, see **Vital Staining.** But the procedure employed by Oliver, J., Bloom, F. and MacDowell, M., J. Exper. Med., 1941, **73,** 141–160 deserves mention because it gives a clear demonstration that the cells of abnormal proximal convoluted tubules can be marked by their inability to concentrate trypan blue which consequently stains the tubule wall diffusely. This is beautifully illustrated in colors. Microscopic observations, having a close relation to function, are easily made on the kidneys of lower forms. See the account of contractility of the ciliated necks of renal tubules in Necturus by Lucas, A. M. and White, H. L., Anat. Rec., 1933, **57,** 7–11.

The study of renal tubules present in tissue cultures is useful up to a certain point in the study of function. Thus Chambers, R. and Cameron, G., Radiology, 1941, **37,** 186–193 have found that susceptibility to x-rays is increased when a secretory stimulant is added but that in cultures it is distinctly less than *in vivo.* See references accompanying this paper.

A method has been devised by Crabtree, C. E., Endocrinology, 1941, **29,** 197–203 of measuring by a differential count the number of Bowman's capsules made of cuboidal as contrasted with squamous cells. The count appears to provide an index of age and sex variations in normal mice and of the influence of testosterone proprionate on castrated mice.

Methods for estimating the distribution of *enzymes* in the tissue components of the rabbit's kidney are given by Weil, L. and Jennings, R. K., J. Biol. Chem., 1941, **139,** 421–432. They depend on

topographic correlation between distribution of cell types in 15 $\mu$ frozen sections and decomposition of substrates. The techniques are capable of demonstrating catheptic, aminopoly-peptidase and esterase activities in all of the epithelial components and of showing that the cells of the proximal and distal convoluted tubules are about twice as active enzymatically as those of the ascending and descending loops of Henle and about 4 times as active as the cells of the collecting tubules. Amylase and dipeptidase activities can also be localized and expressed quantitatively in relative terms.

Techniques capable of revealing very interesting data on the shape of cells of the proximal tubule have been devised and employed by Foote, J. J. and Graffin, A. L., Am. J. Anat., 1942, **70**, 1–20. They can probably be employed to advantage in different functional states and to other than renal cells.

Methods have been elaborated for measurement of the renal filtration surface and data have been supplied for the albino rat (Kirkman, H. and Stowell, R. E., Anat. Rec., 1942, **82**, 373–389). The original paper should be consulted.

pH determinations can be made as described by Emmel, V. M., Anat. Rec. 1940, **78**, 361–377 by means of a capillary glass electrode (Voegtlin, C. and Kahler, H., Science, 1932, **75**, 362) and a vacuum tube potentiometer (Hill, S. E., Science, 1931, **73**, 529). It is significant that increase in acidity of the renal cortex immediately follows ligation of the renal artery and that the mitochondria respond by enspherulation and fragmentation within 6 minutes. The kidney is an organ in which mitochondria must be examined with the utmost promptness. But Fuller, R. H., Arch. Path., 1941, **32**, 556–568 could find no relation in a rather large number of cases studied between age, hours postmortem and cause of death (except renal disease) and quantity and distribution of stainable lipoid.

For application to proximal convoluted tubules in phlorizin glycuresis of the Kabat and Furth procedure for alkaline phosphatase see Kritzler, R. A. and Gutman, A. B., Am. J. Physiol., 1941, **134**, 94–101. See **Phosphatase.**

**King's Carbol-Thionin** stain for Nissl bodies (Addison in McClung, p. 450). Stain paraffin or celloidin sections, 2–3 min., in sat. thionin in 1% aq. carbolic acid. Then wash quickly in aq. dest., differentiate in 95% alcohol. Pass through equal parts absolute alcohol and chloroform to xylol and mount in balsam.

**Kinney's Method** for staining reticulum (Kinney, E. M., Arch. Path., 1928, **5**, 283). Fix 18 hrs. in 1 gm. sodium sulphantimonate dissolved in 100 cc. 4% formalin immediately before using. Imbed in paraffin, but more than 1 or 2 hrs. in xylol or cedar oil will remove the dark brown stain from the reticulum. Hematoxylin is contraindicated as counterstain because it obscures the color of the reticulum. Other ordinary counterstains can be used. This method works well even with autopsy material. It is recommended particularly for kidney and pancreas. Results are sometimes patchy in the spleen.

**Kleinenberg's** fixative. Saturated picric acid in 2% aq. sulphuric acid. Embryos and marine organisms.

**Kolatchew Fluid,** see **Golgi Apparatus.**

**Korff's Fibers** of dentin, see **Teeth, Developing.**

**Kossa,** see his test for **Calcium.**

**Krajian's Congo Stain.** Elastic fibers (Krajian, A. A., Arch. Path., 1934, **18**, 378–380). Fix in 10% formalin, 24 hrs. or more. Cut frozen sections. Wash them in tap water. Place in 2% aq. aluminum chloride 5–10 min. Wash and stain 10 min. in 8 cc. 4% Congo red in 5% aq. sodium citrate + 2 cc. glycerin C.P. After washing in tap water transfer to 1% aq. KI for 10 sec. agitate. After again washing in tap water, stain 5–10 min. in: anilin blue, 1.5 gm.; orange G, 2.5 gm.; resorcinol, 3 gm.; phosphomolybdic acid, 1 gm.; aq. dest., 100. Wash carefully in tap water. Blot sections on slides. Dehydrate in absolute alcohol 2 min.; clear in origanum oil; pass through xylol to balsam. Elastic fibers bright red, fibrin dark blue.

**Krause's End-Bulbs.** Methylene blue demonstration of in skin of forearm (Weddell, G., J. Anat., 1940–41, **75**, 346–367). See **Skin.**

**Krause's Membrane.** Special technique for, see Dahlgren (McClung, p. 427).

**Kronig's Cement** is recommended by Bensleys (p. 41) for ringing preparations mounted in **glycerin jelly** or glycerin: 7–9 parts colophonium (resin) melted and stirred with 2 parts beeswax.

**Kurloff Bodies** are cytoplasmic inclusions which frequently occur in the non-granular leucocytes of guinea pigs. They show particularly well in smears of the spleen, may attain a size equal to that of the nucleus and can be brilliantly colored supravitally by 1:2000 brilliant cresyl blue in physiological salt solution (Cowdry, E. V. chapter in Rivers'book on Viruses, Baltimore, Williams & Wilkins, 1928, p. 141).

**Kultschitzky's Hematoxylin** is 1 gm. hematoxylin dissolved in a little alcohol made

up to 100 cc. with 2% aq. acetic acid (Lee, p. 526).

**Lacteals,** see **Lymphatic Vessels.**

**Lactophenol,** a fixative for **Bilharzial Cercariae.** See Lactophenol-methylene blue technique under **Fungi.**

**Laidlaw's Methods.** 1. For *inclusion bodies* (quoted from Pappenheimer, A. W. and Hawthorne, J. J., Am. J. Path., 1936, **12,** 625–633, see colored figure, who used it for cytoplasmic inclusions in liver cells). Fix in sat. aq. corrosive sublimate 100 cc. + 5% glacial acetic acid or in Zenker's fluid without acetic. Imbed in paraffin, cut sections 3$\mu$. Remove paraffin and pass down to water. Weigert's iron hematoxylin (2%) 5 min. Differentiate in 0.5% acid alcohol. Rinse in tap water, then aq. dest. 1% aq. acid fuchsin 5–15 min. Rinse in aq. dest. Mordant in 1% phosphomolybdic acid 30 sec. Rinse in aq. dest. Differentiate in 0.25% orange G in 70% alc. Dehydrate, clear and mount in balsam.

2. For silver staining of *skin and tumors* (Laidlaw, G. F., Am. J. Path., 1929, **5,** 239–247). Fix in Bouin's fluid or in 10% neutral formalin for 3 days. (To make the Bouin's fluid he uses, add 100 cc. commercial formalin and 20 cc. glacial acetic acid to 300 cc. tap water and saturate with picric acid). Fix paraffin sections to slides by **Masson's Gelatin Glue.** Wash Bouin sections for 20 min. in running water, and formalin ones for 5 min. 1% alc. iodine, 3 min., rinse in tap water. 5% aq. hypo (sodium thiosulphate), 3 min., rinse in tap water. ½% aq. potassium permanganate 3 min., rinse in tap water, 5% oxalic acid, 5 min. Wash in running water, 10 min. Aq. dest. 3 changes in 5–10 min. to clean before adding silver. Heat stock **Lithium Silver** solution to 50°C. and stain in oven for 5 min. Pour aq. dest. over both sides of slides. Flood sections frequently for 3 min. with 1% formalin in tap water. Again rinse both sides of slides with aq. dest. 1:500 yellow gold chloride in aq. dest. in Coplin jar at room temperature, 10 min. Rinse both sides with aq. dest. Pour on 5% oxalic acid 10 min. Rinse in aq. dest. Pour on 5% hypo changing as often as it becomes turbid, 10 min. Wash in running water. Counterstain if desired. Dehydrate, clear and mount in usual way. Reticulum, black threads; collagen reddish purple.

**Lake Ponceau,** see **Ponceau 2R.**

**Lampblack.** A colloidal suspension of lampblack is an excellent substance to inject intravenously to demonstrate phagocytosis, especially by monocytes. McJunkin, F. A., Arch. Int. Med., 1918, **21,** 59–64, advised adding 0.4 gm. of carefully pulverized lampblack to 100 cc. 2% gelatin in aq. dest. Inject intravenously with 5–9 cc. 10% aq. sodium citrate, as in the case of **Higgins' Ink.** The method has been slightly modified by Simpson, M. J., J. Med. Res., 1922, **43,** 77–144; Wislocki, G. B., Am. J. Anat., 1924, **32,** 423–445; and Lang, F. J., Arch. Path., 1926, **1,** 41–63.

**Langerhans,** see **Islets of.**

**Lard,** reactions in tissue to fat stains after various fixations (Black, C. E., J. Lab. & Clin. Med., 1937–38, **23,** 1027–1036).

**Large Intestine.** The conditions that influence the appearance of sections are easier to guard against than in the **Small Intestine** because of the absence of villi and greater uniformity of contents. The pronounced influence of degree of distention is described and well illustrated by Johnson (F. P., Am. J. Anat., 1912–13, **14,** 235–250).

**Lauth's Violet,** see **Thionin.**

**Lead,** histological demonstration.

1. Mallory and Parker's method (Mallory, F. B. and Parker, F. J., Am. J. Path., 1939, **15,** 517–522): Fix tissues in 95 or abs. alcohol (not formalin). Stain celloidin sections at 54°C. in: 5–10 gm. hematoxylin dissolved in few drops abs. or 95% alcohol + 10 cc. freshly filtered 2% aq. $K_2HPO_4$ for 2–3 hrs. Wash changing tap water 10–60 min., dehydrate in 95% alc., clear in terpineol and mount in terpineol balsam. Lead light to grayish blue, nuclei deep blue. Another method applicable to paraffin sections of Zenker fixed material is to stain in 0.1% methylene blue in 20% alc. 10–20 min. Differentiate 10–20 min. in 95% alc., dehydrate, clear and mount. Phloxine is recommended as a contrast stain before the methylene blue.

2. Chromate method (Frankenberger, Cretin). By simply fixing in **Regaud's Fluid** lead is precipitated as insoluble yellow lead chromate easily identifiable microscopically. This method is strongly advised by Lison (p. 101). It has been used by Truc (E., Bull. d'Hist. Appl., 1929, **6,** 393–399). See Sieber (E., Arch. f. exper. path. u. pharmak., 1936, **181,** 273–280) for demonstration of lead in bones.

3. Attempts have been made to identify lead after *microincineration* by exposure to hydrogen sulphide, because lead sulphide is black, but Gordon H. Scott emphasizes difficulty in distinguishing it from other sulphides and from carbon in imperfectly incinerated specimens (McClung, p. 660).

Methods for chemical determination

of lead in biological materials are important as checks on above. Consult Smith, F. L. 2nd., Rathmell, T. K. and Williams, T. L., Am. J. Clin. Path., 1941, **11**, Suppl. 5, 653–668.

For a convenient method of giving *colloidal lead* intravenously to rabbits see Crawford, B. L., Stewart, H. L., Willoughby, C. E. and Smith, F. L., Am. J. Cancer, 1938, **33**, 401–422. The authors describe techniques for direct analysis of lead in the tissues.

**Leather Brown,** see **Bismark Brown Y.**

**Leather Yellow,** see **Phosphine.**

**Lebowich's** soap-wax technique eliminates use of alcohol, xylol and overnight drying of paraffin sections. Takes only 6–8 hrs. (Moritz, C. E., Stain Techn., 1939, **14**, 17–20).

**Lecithin,** a compound of phosphoric acid, glycerol, choline and 2 fatty acid molecules. It is a phosphatide soluble in alcohol, chloroform, ether and benzene, see **Lipoids.**

**Lee-Brown.** Modification of Mallory's aniline blue connective tissue stain (Lee-Brown, R. K., and Laidley, J. W. S., J. Urol., 1929, **21**, 259–274). Mallory (p. 155) states that the following technique is particularly valuable for the kidney. Treat paraffin sections of Zenker fixed material with iodine to remove mercury. Wash. 1% aq. phosphomolybdic acid, 30 sec. Wash in aq. dest. 1–2 min. Stain in: aniline blue, 0.5 gm.; orange G., 2 gm.; phosphomolybdic acid, 2 gm.; aq. dest., 100 cc. for 30 min. at 55°C. Wash in aq. dest 2–5 min. 1% aq. phosphomolybdic acid, 30 sec. 95% alc., abs. alc., xylol, balsam. Glomerular basement membrane and collagen, deep blue; nuclei, orange.

**Leishmania Donovani,** a search for stains that will color more rapidly than Giemsa revealed Astra violet F. F. Extra, Himmelblau, Magenta Lermont and Navy blue shade, each to be used in fresh 10% aq. solution (Takasaki, S., Lues, Tokyo, 1938, **16**, 127).

**Length** measurements:
Millimeters to inches × 0.0394. Inches to mm. × 25.4. See **Micron.**

**Leprosy Bacilli.** Stain by carbol-fuchsin in smears. See **Concentration** method for collecting bacilli from lesions. For study in sections, see **Acid Fast Bacilli.**

**Leptospiras,** method for isolation from water (Bauer, J. H., Am. J. Trop. Med., 1927, **7**, 177–179. See **Spirochetes.**

**Leuco Basic Fuchsin.** To make add to 200 cc. aq. sol. fuchsin, 2 gm. potassium metabisulphite and 10 cc. N hydrochloric acid. After bleaching 24 hrs. add 0.5 gm. Novit, shake 1 min. and filter through coarse paper. Resulting clear solution works nicely in Feulgen technique (Coleman, L. C., Stain Techn., 1938, **13**, 123–124).

**Leuco-Dyes** as vital stains. Make 0.01% aq. solutions of methylene blue, azur A, thionin toluidine blue and brilliant cresyl blue. Add to 100 cc. 1–2.5 cc. N/10 $Na_2S_2O_3$ and 1–4 cc. N/10 HCl. Mix and store at room temperature in dark. To stain, add 1–2 drops of leucobase to the protozoa, blood cells, etc. in physiological saline. Said to give good contrast staining of nucleus and cytoplasm and to be useful in oxidation-reduction determinations (Roskin, G., Arch. Russ. Anat. Hist. Embr., 1937, **16**, 107–109).

**Leucocytes.** In the broad sense they include all white blood cells but the term is generally restricted to the "granular" leucocytes as compared with the "non-granular" ones (**Lymphocytes** and **Monocytes**). In a still narrower sense the leucocytes include only polymorphonuclear neutrophiles, eosinophiles and basophiles which are easily found in circulating blood as contrasted with less differentiated leucocytes called **Myelocytes** and **Myeloblasts** generally confined to the bone marrow.

For mitochondria within leucocytes supravital staining with **Janus green** is indicated. In smears **Giemsa's** stain has a little advantage over **Wright's** in the fact that it better demonstrates any bacteria that may be present. The **May-Giemsa** technique is most used in Europe. It is, in effect, a double staining because the air dried smears are first treated with the **May-Grunwald** combined fixative and stain and are later colored by Giemsa's stain. It gives satisfying deep colors. The **Kardos-Pappenheim** modification is suggested when a particularly intense coloration of neutrophilic granules is desired. **Ehrlich's** triacid stain may likewise be useful because it is said to stain the neutrophilic granules leaving the azur granules untouched.

Leucocytes give strong **Peroxidase** and **Oxidase** reactions, which are, however, not specific for them. The **Golgi Apparatus** (reticular material) can be demonstrated by long treatment with osmic acid or by the Cajal uranium nitrate and silver method (Cowdry, E. V., J. Exper. Med., 1921, **33**, 1–11). The demonstration of degenerative leucocytic changes associated with ageing is described by Lowell (A. L., J. Lab. & Clin. Med., 1937–38, **23**, 791–796), of variability in relation to alterations in meteorologic conditions by Berg (M., J. Lab. & Clin. Med., 1937–38, **23**, 797–803) and of lipoid components by Bacsich (P., J. Anat.,

1935–36, **70**, 267–272). *Chemotactic response* and motility can be measured both in tissue cultures (Coman, D. R., Arch. Path., 1940, **30**, 896–901) and directly by observing the behavior of leucocytes with relation to bacteria and in temporary mounts (Mallery, O. T. and McCutcheon, M., Am. J. Med. Sci., 1940, **200**, 394–399). By the latter method differences in behavior of neutrophiles from seriously ill and normal persons have been reported. Motion pictures are of great assistance in making a thorough analysis of the movements and behavior of leucocytes. Some excellent ones, taken by Dr. W. H. Lewis, are available for distribution by the Wistar Institute of Anatomy in Philadelphia. To investigate their behavior after they have left the blood vessels and entered the surrounding tissues is immensely more difficult. The only method that gives promise of important results is to employ for this purpose special chambers inserted in the ears of rabbits (Clark, E.R. and E. L., Am. J. Anat., 1936, **59**, 123–173). See **Neutrophile, Eosinophile** and **Basophile Leucocytes.**

**Leucocyte Counts.** 1. *Total* number white blood cells per c. mm. Over 12,000 a leucocytosis, less than 5000, a leucopenia. Average about 7,500.

2. *Differential.* Smears colored by **Giemsa's** or **Wright's** stains are more satisfactory than supravitally stained preparations because the latter are more difficult to handle and the cells are slowly dying and showing more and more deviations from normal. Relative number of different white cells is expressed in percentages, i.e. neutrophiles 55–75, eosinophiles, 2–4, basophiles, 0–1, lymphocytes 21–31, and monocytes 4–5. Both total and differential counts should be correlated to avoid misconceptions. 60% neutrophiles in total count of 8,000 amounts to 4,800 neutrophiles per c. mm. 80% neutrophiles in total count of 4,800 is the same, namely 4,800 neutrophiles per c. mm. although a relative neutrophilic leucocytosis exists. 60% neutrophiles in a total count of 16,000 makes on the other hand 9,600 neutrophiles per c. mm. which is an actual neutrophilic leucocytosis. 20% lymphocytes of 9,000 is the same number per c. mm. as 60% of 3,000; while 30% of 11,000 is an actual lymphocytosis.

3. *Age.* Since young neutrophiles have fewer nuclear lobes than older ones counts of the number with from 1–5 lobes were made by Arneth. Today simpler methods are used.

The Schilling is the usual one. It is both a total, a differential and an age count combined. The normal is given

| | | |
|---|---|---|
| Total | 5,000 to 10,000 | |
| B | 0–1 | |
| E | 2–4 | |
| M | 0 | Leucocytes |
| J | 0–1 | |
| St | 3–5 | |
| S | 51–67 | |
| L | 21–35 | |
| Mon. | 4–5 | |

above. B = basophile. E = eosinophile. M = myelocyte (Nucleus large, occupying about half cytoplasmic area, spherical to oval or kidney-shaped, pale staining, chromatin reticulated, nucleoli present. Cytoplasm faintly basophilic with few specific granules which are small, often difficult to stain and irregularly distributed). J = juvenile (A little larger than mature neutrophiles. Nucleus saucer to bean shaped. Stains poorly. Circumscribed basophilic nucleoli). St = stab nuclear (Slightly smaller than juveniles. Nucleus T V or U shaped but not divided into segments by filaments and without nucleoli). S = segment nuclear (Fully differentiated neutrophiles having 2–5 or more segments often joined only by filaments. Nuclei stain intensely.) L = lymphocyte. Mon = monocyte.

When the numbers of M. J. St. are increased relative to S., it is called a "shift to the left", meaning that immature leucocytes are called into the circulation, which is an unfavorable sign. When the relative number of S is increased over the others, it is termed a "shift to the right", meaning that only mature leucocytes are called out, which is a favorable sign if it follows a previous shift to the left. Details are given by Wintrobe, M. M., Clinical Hematology, Philadelphia, Lea & Febiger, 1942, 792 pp. For blood containing gum acacia, see Monke, J. V., J. Lab. & Clin. Med., 1940–41, 26, 1664–1667 and for interference by decreased fragility of erythrocytes see Bohrod, M. G., J. Lab. & Clin. Med., 1940–41, 26, 1953–1955.

A better method, unfortunately not widely employed, is the filament-nonfilament count. Filaments are neutrophiles in which the nuclear segments are connected by delicate strands apparently made up of nuclear membrane only and nonfilaments are those in which the connections are so wide that they can be resolved into nuclear membrane plus nuclear contents. In 100 neutrophiles there are normally 8–16 nonfilament cells. A greater per cent is a shift to the left. For counts see Krusen,

F. H., Am. J. Med. Sci., 1937, **193**, 470–474.

**Leucocytes.** Developmental series. The technique employed apparently makes a great deal of difference in the conclusions reached. See Cowdry's Histology, p. 99.

1. Maximow and Bloom employing mainly permanent preparations list:

*Hemocytoblasts:* ". . . large (up to 15) ameboid, non-granular basophil cells of lymphoid nature." Occur extravascularly.

*Promyelocytes:* "The oval or kidney-shaped, clear nucleus contains a loose chromatin network and several nucleoli. At the indentation of the nucleus there is a distinct cytocentrum. The ameboid protoplasm is slightly basophil, although it often shows acidophil areas." Specific granules "are scarce and usually confined to the periphery of the cytocentrum and to the acidophil spots in the cell body." Azurophil granules are present but later disappear. They often show mitosis.

*Myelocytes:* "The protoplasm becomes diffusely acidophil while the specific granules increase in number and fill the whole cell body, except for the cytocentrum. The nucleus keeps its compact form while its previously loose chromatin network becomes coarser and stains darker. The nucleoli are indistinct. Mitoses are common."

*Metamyelocytes:* After an unknown number of mitoses a generation appears. The nucleus "as soon as it is reconstructed after the last mitosis, shows a beginning polymorphism and has the shape of a horse-shoe." The mature leucocyte is formed from these cells by individual maturation without division.

2. Sabin and associates relying chiefly on supravital stains list:

*Reticular cells:* They "are small, their cytoplasm is faintly basophilic, as seen in fixed films, and in supravital preparations they show no differentiation of specific substances." Reticular cells "lack the striking rod-shaped mitochondria which characterize the lymphocytic strain. . . . The nuclei have less sharp contours and less chromatin than those of lymphocytes."

*Myeloblasts:* These differ "through the elaboration of a marked basophilia and of great numbers of small mitochondria. . . . In supravital technique, the myeloblast has usually no stainable substance except mitochondria . . ." but occasionally a few vacuoles reacting to neutral red are present as well as some which are not colored by it.

*Myelocytes A:* The earliest stage with the specific granules up to 10 "reacts with a single blue granule in the oxydase test."

*Myelocytes B:* "May be conveniently divided into those with less than half and those with more than half the full quota of granules."

*Myelocytes C:* These cells contain the full quota. *Metamyelocytes:* They "show the earliest signs of the nuclear changes toward polymorphism and the first sign of the transformation of the cytoplasm to a phase sufficiently fluid to allow the flowing of granules which is essential for ameboid movement. In passing through these stages, there is a gradual decrease of basophilia of the cytoplasm and in the numbers of mitochondria. The basophilia disappears entirely in the early leucocytes, while the mitochondria persist in small numbers until the stage of senility in the leucocytes."

**Leucocytic Infiltrations.** A convenient way to produce an intense local neutrophilic infiltration is to inject starch as described by Chambers, R. and Grand, C. G., Am. J. Cancer, 1937, **29**, 111–115. Cowdry, E. V. and Ruangsiri, C., Arch. Path., 1941, **32**, 632–640 made repeated injections of 1% corn starch suspensions in physiological saline in amounts of 0.1–0.2 cc. into leprous nodules of rats.

**Leucocytozoa,** Protozoa, belonging to the Hepatozoidae, which inhabit the monocytes of dogs, rats, and other animals particularly in the tropics. See, Wenyon, C. M., Protozoology. New York: William Wood & Co., 1926, 2,1053–1563.

**Leucosin,** a stored reserve in lower plants (Taylor in McClung, p. 221).

**Levulose Syrup** for fluid mounts. Mallory (p. 99) specifies 30 gms. levulose dissolved in 20 cc. water by warming at 37 °C. for 24 hrs.

**Lewis-Locke** solution, see **Locke-Lewis.**

**Lieberkühn's Glands,** data on size, surface area, number of cells etc. in human large intestine (Policard, A., Bull. d'Hist. Appl., 1939, **16**, 261–262).

**Liebermann-Burchardt** reaction for *cholesterol* and its esters (*cholesterides*).

1. Modification of A. Schultz. Expose frozen sections of formalin fixed tissue at least 4 days (more in winter) to strong light, if possible sunlight. Mount. Dry carefully with blotting paper. Cover with few drops equal parts acetic and sulphuric acids. Drain and examine in the reagent. Cholesterol and its esters dark blue or red purple becoming green.

2. Modification of Romieu (M., C. rend. Acad. d. Sci., 1927, **184**, 1206–1208) Mount frozen sections of formol or Bouin (less acetic) fixed tissues and dry. Cover with 1 drop conc. sulphuric acid,

3–15 sec. Stop reaction by adding 2–3 drops acetic anhydride. Wash with several drops of same. Cover and examine immediately. Cholesterol and its esters violet lilac or red purple, becoming green. The above two methods abbreviated from Lison (p. 210) are in his excellent judgment specific for cholesterol and its esters if positive. A negative reaction does not definitely prove their absence. See Swyer, G. I. M., Cancer Research, 1942, 2, 372–375 for quantitative measurement of the color.

**Light Blue,** see **Spirit Blue.**

**Light Green,** see **Methyl Green.**

**Light Green N,** see **Malachite Green.**

**Light Green SF** yellowish (CI, 670) S—acid green, fast acid green N—Commission Certified. This acid di-amino triphenyl methane dye is a sulfonated derivative of brilliant green and a valuable counterstain for safranin. It is used by Twort, F. W., Brit. J. Exp. Path., 1924, 5, 350–351 as a double stain with neutral red for animal parasites and microorganisms in tissues. Unfortunately light green fades quickly. Conn (p. 110) recommends fast green FCF as a substitute.

**Lighting,** see **Illumination.**

**Lignin Pink,** a monazo acid dye (British Drug Houses Ltd.). Advised 0.5% aq. solution as a chitin stain and a contrast stain with chlorazol Black E (Cannan, H. G., J. Roy. Micr. Soc., 1941, 61, 88–94).

**Lilienfeld-Monti** test for phosphorus is not a satisfactory microchemical method. See Bensley's method (R. R., Biol. Bull., 1906, 10, 49–65) and criticism by Lison (p. 118).

**Lillie's** chrom-osmic-acetic fixative. ½% aq. chromic acid, 15 cc.; 2% aq. osmic acid, 3.5 cc.; glacial acetic acid, 3 drops. Used by him for echinoderm eggs.

**Line Test** for vitamin D. This is the basis for calculating the U.S.P. unit of vitamin D potency. The line test was apparently first introduced by McCollum, E. V., *et al.*, J. Biol. Chem., 1922, 51, 41–49. A critique of the test is given by Bills, C. E., *et al.*, J. Biol. Chem., 1931, 90, 619–636. See also Sherman, H. C., The Chemistry of Food and Nutrition, New York: MacMillan, 1941, 611 pp. A slightly modified technique is proposed and given in detail by Martin, G. J., J. Lab. & Clin. Med., 1940, 26, 714–719. Inject rats intraperitoneally with 1 cc. 1% aq. sodium alizarin sulfonate at pH 8.0 and give supplements of measured amounts of vitamin D orally. Animals similarly stained but not given the vitamin serve as controls. After test periods of 1 or 2 days, kill the animals, remove radii and ulnae and examine grossly and microscopically for alizarin stained lines at epiphysis. See also use of **Alizarin Red S.** Both this and the sulfonate are better than **Madder** because they provide quicker and more intense coloration of bony calcium laid down during the period that they are available in the circulation as accelerated by vitamin D.

**Linguatulidae,** see **Parasites.**

**Linin** (L. linum, flax). The acidophilic, thread-like framework of nucleoplasm seen in sections but not in the living nucleus.

**Lipase.** Frozen sections 30$\mu$ thick and 4.5 mm. in diameter of beef adrenals are extracted in 30% glycerol + equal volume 1% methyl butyrate in glycine — NaOH buffer at pH 8.7; digested at 40°C.; enzyme action arrested by addition of 2% phenol (10 parts) and 0.04% brom-thymol blue (1.5 parts) to 3.5 times total volume; and end point titrated at pH 6.5 with 0.05 N HCl. This point is determined by comparing color with standard color of brom-thymol blue in phosphate buffer pH 6.5. Nearby sections, some stained with hematoxylin and eosin, and others, with Sudan III, are examined histologically. The medulla, which exhibits most lipolytic activity, contains least lipid. Estimations of esterase are also described by Glick and Biskind (D. and G. R., J. Biol. Chem., 1935, 110, 575–582). See Barnes, J. M., Brit. J. Exp. Path., 1940, 21, 264–275 for analysis of lipase in lymphocytes and polymorphonuclear leucocytes and Hoagland, C. L., et al., J. Exper. Med., 1942, 76, 163–173 for lipase determinations in elementary bodies of vaccine virus.

**Lipids.** Identification of various kinds in microscopic preparations is extremely difficult. As Lison (p. 192) has shown, reliance cannot be placed in *solubility tests.* Some bodies, soluble in alcohol, ether, chloroform, carbon tetrachloride and so on, are not fats while some fats show considerable resistance to such solvents. Formalin fixation itself causes marked changes in solubility of fatty bodies (Kaufmann, C. and Lehmann, E., Virchow's Archiv. f. Path. Anat. und Physiol., 1926, 261, 623–648). It is not unusual to find fats slightly soluble or insoluble in microscopic preparations which on chemical extraction are soluble. Results of examination in *polarized light* must, he states, be interpreted with caution. Glycerides and fatty acids examined *in vivo* are never birefringent in the dissolved condition. After freezing or treatment with formalin they can become crystalline and birefringent.

Cholesterol, in the form of birefringent, rhombic plates, is of rare occurrence *in vivo*, but easily recognizable. Cholesterides appear sometimes as droplets presenting the black cross of polarization when viewed at low temperature. When temperature is increased they lose birefringence and look like droplets of fat. Birefringence is lost as a result of osmication. Coloration with sudan and mounting in syrup of levulose decreases birefringence. Lison gives following tabular method of analysis (abbreviated).

1. In frozen sections, mounted in levulose syrup, without artificial coloration, generally yellow orange or brown.
   2. Iodine - iodide solution (like Gram's or Lugol's) gives black-green or brown. Chromic acid solution decolorizes quickly or slowly—*carotinoids.*
   2. Above reactions negative. Sulphuric acid sometimes gives red color—*chromolipoids.*
1. In frozen sections show no natural color.
   2. Liebermann reaction (Schultze or Romieu technique) positive: color blue, purple or violet, becoming green.
      3. Digitonine reaction (Brunswick or Leulier-Noel technique) gives crystals strongly illuminated between crossed nicols, unstainable by histological methods—*free cholesterol.*
      3. Digitonine reaction gives no crystalline ppt.—*cholesterides.*
   2. Liebermann reaction negative after repeated attempts, no coloration or brown or red color.
      3. Mounted in levulose syrup, without artificial coloration, examined with crossed nicols, brightly illuminated and showing cross of polarization—*Lipines.*
      3. Mounted in same way, without artificial coloration, examined with crossed nicols, not illuminated or illuminated but without showing cross of polarization.
         4. Smith-Dietrich reaction at 50°C. positive, color black—*Lipines.*
         4. Above reaction negative. Coloration gray or absent.
            5. Lorrain Smith reaction with nile blue sulphate: rose—*non-saturated glyceride.*
            5. Above reaction absent or blue—*Saturated or non-saturated glyceride, or fatty acid or Lipine.*

The much used **Osmic Acid** and **Sudan** staining methods are helpful when other evidence is available as to chemical constitution of substances demonstrated. See **Fatty Acids, Soaps, Neutral Fats** (Glycerides), **Lipoids, Cholesterol** (free), **Cholesterol Esters, Myeloidin, Myelin,** etc.

**Lipines,** see **Lipoids.**

**Lipiodol,** reactions in tissue to fat stains after various fixations (Black, C. E., J. Lab. & Clin. Med., 1937-38, **23,** 1027-1036).

**Lipochrin** is the term applied to certain usually solitary fatty droplets present in retinal cells of several vertebrates but absent in guinea pigs and man. For literature see Arey, L. B. in Cowdry's Special Cytology, 1932, **3,** 1219.

**Lipochrome.** Defined by Lison (p. 244) as a solution of a carotinoid in a fatty body, the latter by itself uncolored, often found in nerve, hepatic, cardiac muscle cells and elsewhere. See **Carotinoids.**

**Lipofuscins** are fats colored by the carotene dissolved in them found in nerve, hepatic and cardiac muscle cells (Mallory, p. 125).

**Lipoids** (G. *lipos,* fat + *eidos,* appearance). This term is taken to mean almost anything even remotely looking like fat. Generally included under it are lecithin, cephalin, sphingomyelin, kerasin, phrenosin, etc. which cannot be identified microchemically in sections. They are referred to as **Lipines** by Lison. See his tabular analysis under **Lipids.** See methods of **Ciaccio** and **Smith-Dietrich.**

**Lipolytic Enzymes,** see **Lipase.**

**Lipomicrons,** small droplets of lipid in circulating blood. See **Chylomicrons.**

**Lison's** glycogen method (Lison, p. 227). Fix in dioxan saturated with picric acid, 8.5 parts; formalin, 1 part; and acetic acid, 0.5 cc. Pass direct through dioxan, dioxan-paraffin, paraffin, imbed, section and stain in the usual way.

**Lithium Carmine** 1. To make Orth's lithium carmine dissolve 2.5-5 gms. carmine in 100 cc. sat. aq. lithium carbonate. Boil for 10-15 min. and, when cool, add a crystal of thymol as an antiseptic. Stain sections about 3 min. Differentiate in **Acid Alcohol.** Wash in water, dehydrate in alcohol, clear in xylol, or toluol, and mount in balsam. Gives sharp bright red stain of nuclei often useful in place of the blue of hematoxylin, of methylene blue, etc. It may be used after almost any good fixative.

2. Lithium carmine has also been employed in many classical experiments as a vital stain (Aschoff, L. and Kiyono; K., Folia Haemat., 1913, **6,** 213; Suzuki, T., Nierensekretion, Jena, 1912; Kiyono, K., Die Vitale Karminspeicherung, Jena, 1914, etc.). Filter a sterilized concentrated suspension of carmine rubrum optimum (5 gm.) in cold sat. aq. lithium carbonate and slowly inject 5-10 cc. intravenously in rabbits (Foot, McClung, p. 115). The Bensleys (p. 151) give the following directions.

Cook on water bath 100 cc. sat. aq. lithium carbonate + 5 gm. carmine rubrum (Grubler) for ½–1 hr. Filter hot. Allow to settle and cool. Filter cold. Sterilize in autoclave and filter again through sterile filter. Inject intravenously once or more. Kill the animal and fix tissues in alcohol, formalin or formalin-Zenker.

**Lithium Silver** of Hortega as described by Laidlaw (G. F., Am. J. Path., 1929, **5**, 239–247): In 250 cc. glass stoppered bottle dissolve 12 gms. silver nitrate, C.P. in 20 cc. aq. dest. Add 230 cc. sat. lithium carbonate, C.P. in aq. dest. Shake well. Let settle to about 70 cc. ppt. Decant. Wash ppt. with aq. dest. 3 or 4 times. Decant all except 70 cc. ppt. Add ammonia water (26–28%) shaking until fluid is nearly clear. Add aq. dest. to total vol. of 120 cc. Filter through Whatman filter paper No. 42 or 44 or Schleicher and Schüll No. 589 into stock bottle. See **Laidlaw's Methods.**

**Litmus** as a vital indicator of acidity and alkalinity in rats and mice (Rous, P., J. Exper. Med. 1925, **41**, 379–397).

**Liver.** In this very large organ, as in the lungs, it is necessary to carefully select the specimens excised for study. It is bad practice to take only slices vertical to the surface including the capsule. The deeper parts should be included. How the weight and structure of the human liver varies with phases of assimilation and secretion as in rabbits (Forsgren, E., Act. med. Scandin., 1931, **76**, 285–315) and in rats (Higgins, G. M., Berkson, J. and Flock, E., Am. J. Physiol., 1933, **105**, 177–186) remains to be determined. Effect of different dehydration and clearing agents on liver (Ralph, P., Stain Techn., 1938, **13**, 9–15).

**Locke-Lewis** solution. NaCl, 0.85 gm.; KCl, 0.042 gm.; $CaCl_2$, 0.025 gm.; $NaHCO_3$, 0.02 gm., dextrose, 0.01–0.25 gm.; aq. dest., 100 cc. Should be freshly made. Owing to presence of $NaHCO_3$ must not be sterilized by heat.

**Loeffler's Alkaline Methylene Blue.** As emended Soc. Am. Bact. A. Methylene blue (90% dye content) 0.3 gm. + 95% ethyl alcohol, 30 cc. B. 0.01% aq. KOH by weight 100 cc. Mix A and B (McClung, p. 137).

**Loose Connective Tissue.** Subcutaneous tissue of this sort is often chosen for investigation. It may be dissected out and spread on slides. A good way, demanding practice, is to tease the tissue apart, without the addition of any saline solution, so that one edge is parallel to the end of the slide and about 4 cm. from it. This edge is allowed to dry and become affixed to the slide, while the remainder of the tissue is kept moist and is stretched with needles evenly along the length of the slide into a fairly thin film. This spread is then examined in the fresh state, with various solutions added, or it is fixed and stained like a blood smear. Separation of components into a sufficiently thin spread is facilitated by first making a bulla (L. for bubble) under the epidermis by the local injection of fluid (salt solution, serum, etc.).

Sylvia H. Bensley (Anat. Rec., 1934, **60**, 93–109) employed a graphic method for demonstration of ground substance. She adapted a culture of paramoecia to 0.6–0.8% salt solution, injected subcutaneously into a guinea pig, excised the bulla and examined it as a whole mount with cover glass supported at edges. Actively motile organisms suddenly rebounded without coming into contact with microscopically visible structure and none escaped into the surrounding fluid from the bulla. This is evidence of the existence in loose connective tissue of an amorphous ground substance in the physical condition of a gel. She described, and used to advantage, methods for determination of the refractive index, consistency, digestability and tinctorial properties of this substance in several parts of the body.

Methods for the identification of **Collagenic** and **Elastic Fibers, Fibroblasts, Tissue Basophiles** and other constituents are described under the respective headings. See also **Tissue Fluid.**

**Lorrain Smith,** see **Nile Blue Sulphate.**

**Lucidol,** a trade name for benzoyl peroxide.

**Lucite,** disadvantages of as substitute for Canada balsam (Richards, O. W. and Smith, J. A., Science, 1938, **87**, 374). It is used in place of **Quartz** for transillumination by Williams, R. G., Anat. Rec., 1941, **79**, 263–270, and in making containers for museum specimens by Snitman, M. F., Arch. Otolaryng., 1942, **36**, 220–225.

**Lugol's Iodine.** Potassium iodide, 6 gm.; iodine, 4 gm.; aq. dest., 100 cc.

**Luminol** (3-aminophthalhydrazide) made by Eastman Kodak Co. has a marked affinity for hematin yielding brilliant luminescence in ultraviolet light. Hematin in a dilution of 1:100,000,000 can be detected thereby. This is a medicological test of great sensitivity but is not limited to human blood (Proescher, F. and Moody, A. M., J. Lab. & Clin. Med., 1938–39, **24**, 1183–1189).

**Lungs.** To excise properly pieces for fixa-

tion requires great skill especially if lesions are present. The slices should be cut with the sweep of a particularly sharp knife to minimize squeezing and the resultant distortion and displacement of fluids when these are present. The contents of small cavities and bronchi may escape unless care is taken to retain them by immediate coagulation by fixation. Owing to regional differences it is important to select representative areas. To demonstrate the fibrin often present in lesions, **Weigert's** stain is recommended.

Observation of lung through thoracic window *in vivo* (Terry, R. J., Science, 1939, **90**, 43–44), see **Celluloid Corrosion** preparations, **Alveolar Pores.**

**Lymphatic Vessels.** There are many ways of demonstrating lymphatic vessels. The most convenient is to sit in an easy chair and view the splendid moving picture prepared by Dr. Richard L. Webb of the Department of Anatomy of the University of Illinois College of Medicine entitled: "Mesenteric lymphatics, their conduct and the behavior of their valves in the living rat".

Another easy method is to watch absorption of cream in a cat. A fasting animal is fed ½ pint of cream and the abdominal cavity is opened under ether anesthesia a few minutes later. At first sight it may be difficult or impossible to see any lymphatics in the mesentery although a few bean shaped lymph nodes are visible near its base and can be easily felt. Keep the abdominal contents moist with saline. Close the opening. In a little while, when again examined, the lymphatic vessels will be clearly marked in white by the milk fat which has been absorbed by the lacteals and is being transported in them.

A simple method to visualize the pathways of lymphatic drainage from the nasal mucous membrane has been described by Yoffey, J. M., Lancet, 1941, 1, 529–530. Anesthetize a cat. Drop into each nostril 1 cc. 5% trypan blue (T. 1824) in physiological saline (0.85% aq. NaCl). T. 1824 is specified because it is a trypan blue isomer which is deeply colored even in high dilutions but any good trypan blue will do. Dissect away the side of the neck. Lymphatic vessels, deeply stained, will be seen from the nose and pharynx converging to the deep cervical node and from the posterior border of this node a single deep cervical vessel takes origin and proceeds downward in the neck. The technique delineates a functioning system of vessels actually at work.

Lymphatic vessels and capillaries constitute a drainage system provided in largest measure beneath the external surface of the body and the invaginations of this surface into it in the respiratory, alimentary and urinogenital systems. They are absent in the brain and bone marrow and rare or absent in skeletal muscle. See detailed information concerning the organ or tissue, in which it is desired to demonstrate them, to be found in Drinker, C. K. and Yoffey, J. M., Lymphatics, Lymph and Lymphoid Tissue. Harvard Univ. Press, 1941, 406 pp.

Methods for the injection of lymphatics involve forcing fluid containing particulate matter into areas where there are many lymphatic capillaries. A technique for the observation *in vivo* of the superficial lymphatics of human eyelids is described by Burch, G. E., Anat. Rec., 1939, **73**, 443–446. 0.02 cc. of a dilute solution of patent blue V is injected intradermally 5–10 mm. beyond the middle of the lid margin. The lymphatics are apparent in about 5 min. and may be observed as long as 75 min. Consult earlier experiments with this dye by McMaster, P. D., J. Exp. Med., 1937, **65**, 347–372.

A good way is to utilize the transparent ears of white mice to inject the lymphatics with hydrokollag by means of a microdissection apparatus (Pullinger, B. D. and Florey, W. H., Brit. J. Exp. Path., 1935, **16**, 49–61). But the best available technique is closely to examine over long periods of time living non-injected lymphatics in **Sandison** chambers in the ears of rabbits (Clark, E. R. and E. L., Am. J. Anat., 1937, **62**, 59–92.

**Lyons Blue,** see **Spirit Blue.**

**Lymphocytes.** There is no specific stain for lymphocytes, but identification is usually easy at least for small lymphocytes. To observe *motility*, mount fresh blood and ring with vaseline to prevent evaporation. Movements usually begin after the neutrophiles have become active. Examination in the darkfield may be helpful. Mitochondria can be demonstrated easier in lymphocytes by supravital staining with **Janus Green** than in polymorphonuclear leucocytes because they are not obscured by the specific granulations. In the study of smears the characteristic cytoplasmic basophilia of lymphocytes can be brought out by most of the usual stains (**Giemsa's, Wright's**). The **Peroxidase Reaction** of lymphocytes is negative, or very strictly limited. Methods demonstrating **Cathepsin, Nuclease, Amylase, Lipase, Lysozyme** and **Adenosinase** in lymphocytes are de-

scribed by Barnes, J. M., Brit. J. Exp. Path., 1940, 21, 264–275. To determine the age of lymphocytes is extraordinarily difficult. Perhaps the nearest approach to this goal is the work of Wiseman, B. K., J. Exper. Med., 1931, 54, 270–294.

**Lysis.** In histology this term means the solution of a cell resulting from injury to the cell membrane. A choice may be made of several agents productive of this change. As classified by Danielli (Bourne, pp. 74–75) antibodies and polyhydroxylic phenols probably act almost wholly on the *protein* component of the membrane; lipoid solvents, lecithinase, digitonin, sodium or potassium salts of fatty acids and paraffin sulphonates mainly on the *lipoid* part; and the heavy metals probably on both. He suggests the probable modes of action. It is therefore possible that these lytic agents may in their action provide clues as to the nature of the plasma membrane. See **Cell Membranes.**

**Lysozyme.** A method for analysis of lysozyme in lymphocytes and polymorphonuclear leucocytes (neutrophiles) is given by Barnes, J. M., Brit. J. Exp. Path., 1940, 21, 264–275.

**Lyssa Bodies** are small Negri bodies which look optically hyaline, see **Negri Bodies.**

**Maceration** (L. *macerare*, to soak) is a very important technique by which tissues are soaked for considerable periods of time in various fluids which loosen the connections between the cells and allow them to be easily separated for microscopic study. This is a method employed by the great masters in histology which is unfortunately not sufficiently used now-a-days.

For *nervous tissue* Addison (McClung, p. 439) recommends Gage's dissociator which is 2 cc. formalin in 1000 cc. physiological salt solution for 2 or 3 days. After this treatment large ventral horn nerve cells can easily be dissected out with the aid of a binocular microscope, stained with carmine, picrocarmine or a dilute anilin dye and viewed as units with parts of their processes attached.

*Smooth muscle* of the bladder is well dissociated by 10–20% nitric acid (Dahlgren, in McClung, p. 423). The resulting fibers are suitable for class use.

*Thyroid follicles* are freed from the surrounding tissue and can be examined individually after maceration in conc. hydrochloric acid 3 parts and aq. dest. 1 part for about 24 hrs. and thorough washing in at least 10 changes of tap water (Jackson, J. L., Anat. Rec., 1931, 48, 219–239).

*Epidermis* can be separated from dermis by maceration in 1% acetic acid, see **epidermis.**

*Kidney tubules.* Pieces of kidney fixed in 10% formalin or in Kaiserling's solution are placed in conc. hydrochloric acid at room temperature until they become sufficiently softened after 2–7 days. The time depends upon size of piece, degree of fibrosis and other factors. There is no advantage in using fresh tissue. When adequately macerated the almost diffluent tissue is washed in repeated changes of aq. dest. in which it may be kept for several days. Dissect out individual tubules with the aid of a binocular microscope (Oliver, J. and Luey, A. S., Arch. Path., 1934, 18, 777–816).

*Seminiferous tubules.* Whole human testicles are fixed in formalin. They are then cut into segments 1 cm. thick parallel to direction of the lobules. The tunica vaginalis is not removed but is slit through in one or two places with a razor. Each segment is placed in conc. hydrochloric acid, 75 cc., aq. dest. 25 cc. 1–7 days. Heat just below boiling 20–30 min. Tissue shrinks, turns dark brown and softens. A sediment collects in the dish. Part of acid is drawn off with a pipette, boiled water is added and the process is repeated until practically all of the acid is removed. The water is boiled to prevent formation of air bubbles along the tubules. It turns the tubules a yellowish white color in which condition they should be isolated by careful teasing. When the tubules cannot be easily lifted away from one another, the maceration is insufficient. When, on the other hand, they break it is a sign of over maceration (Johnson, F. P., Anat. Rec., 1934, 59, 187–199). A similar method was used by Johnson in 1916 to separate the lobules of the pig's liver.

*Bone cells and lamellae.* Treat a thin bone section with conc. nitric acid as long as 24 hrs. Mount on a slide and squeeze out bone cells by pressure on cover glass. The lamellae can be pealed off easily from a piece of decalcified bone which has been gently boiled in water (Shipley, in McClung, p. 348).

*Enamel rods.* A piece of dental enamel is dissociated with 5–10% hydrochloric acid for 24 hrs. When it has become soft, remove a little with a needle to a slide and tease out. Mount in physiological salt solution under a cover glass. Draw through a little carmine stain with a blotter and wash it out with 10% acetic acid. The specimen can be ringed with paraffin

(Churchill, and Appleton, in McClung, p. 372).

*Nerve cells.* Pieces of gray matter of ventral horn are soaked for 2 or 3 days in 0.02 formalin. The tissue softens, the cells are dissected out and stained with carmine or picro-carmine (Addison, in McClung, p. 439).

**MacNeal's Tetrachrome** is a blood stain containing eosin, methylene azure A, methylene blue and methylene violet. It is employed like Wright's stain. For details see MacNeal, W. J., J. A. M. A., 1922, 78, 1122, and Conn, H. J., Stain Technology, 1927, 2, 31.

**Macrophages.** These are the free cells of the reticulo-endothelial system. Almost any method of exposure to relatively non-toxic, finely particulate matter is sufficient to bring them out. The simplest way is to inject mice with trypan blue as described under **Vital Staining** and to look for the macrophages in spreads of **Loose Connective Tissue.** Another method, used by Maximow, is to give rabbits intravenous injections of saccharated iron oxide or India ink and to examine blood from right ventricle in smears (see Cowdry's Histology, p. 69). Lines of division between macrophages and monocytes, if they exist, are difficult to establish. Supravital staining with **Neutral Red** and **Janus Green** is useful to demonstrate neutral red granules and mitochondria respectively.

**Madder Staining** of bone. Madder is a red dye, prepared from the plant *Rubia Tinctorum* which has been used for thousands of years. Alizarin and purpurin, formed from it, are now made synthetically. Madder should be employed for the vital staining of growing bone as described by Macklin (C. C., Anat. Rec., 1917, 12, 403–405; J. Med. Res., 1917, 36, 493–507). Young rats are suggested as material. Each should eat 1–5 gms. of madder, thoroughly mixed with its food, daily. The calcium deposited in the growing bone while madder is thus made available in the circulation is colored red. Staining is noticeable after 1 day but the feeding should be continued for a week or more.

The ventral ends of the ribs and the epiphyseal lines of long bones are most intensely colored. The bones selected are fixed in 10% neutral formalin, washed and cleaned in water, dehydrated thoroughly in alcohol, placed in benzene for 24 hrs., cleared in oil of wintergreen by the method of **Spalteholz** and examined with binocular microscope as whole objects.

Chemistry of madder staining is discussed by Dr. Richter (Biochem. J., 1937, 31, 591–595). The substance giving the intense carmine red color is apparently purpurin carboxylic acid. Madder is one of the most classical of stains. Its history extends back through the centuries and has been well reviewed by F. T. Lewis (Anat. Rec., 1942, 83, 229–253). See **Line Test.**

**Magdala Red** (CI, 857)—naphthalene pink, naphthalene red, naphthylamine pink, sudan red—According to Conn (p. 102) this basic naphtho-safranin differs from commercial magdala red which is an acid dye belonging to an entirely different group. He calls attention to its use by Kultschitzky, N., Arch. f. Mikr. Anat., 1895, 46, 673–695) in staining elastic tissue of the spleen.

**Magenta,** see **Basic Fuchsin.**

**Magenta II** is triamino ditolyl-phenylmethane chloride probably present in most samples of **Basic Fuchsin.** See **Pararosanilin** (Magenta O), **Rosanilin** (Magenta I) and **New Fuchsin** (Magenta III).

**Magnesium,** Titan yellow method for determination of small amounts in body fluids (Haury, V. G., J. Lab. & Clin Med., 1938, 23, 1079–1084).

Methods for detection in plant cells (Broda, B., Mikrokosmos, 1939, 32, 184). (1) Triturate 1 part quinalizarin with 5 parts sodium acetate crystals. Make to fresh 0.5% solution in 5% aq. NaOH. Addition of 1–2 drops to paraffin section, then 1–2 drops 10% NaOH results after some hours in blue stain. (2) Add to paraffin section 1–2 drops 0.2% aq. Titan yellow, then 1–2 drops 10% NaOH gives rise to brick red stain of magnesium. (3) Add to paraffin section 0.1% aq. azo blue. Gives, without the NaOH, a violet stain of magnesium. An attempt should be made to adjust these techniques to human tissues in which a magnesium salt has been injected.

By means of a specially constructed electron microscope Scott and Packer (G. H. and D. M., Anat. Rec., 1939, 74, 17–45) have accurately localized magnesium and/or calcium in muscle. The method can be extended to other tissues and perhaps to other minerals. Histospectrography gives data on the amount of magnesium relative to the other minerals in the skin of normal and neurodermatitis patients. In the latter there is a magnesium deficiency (MacCardle, R. C., Engman, M. F., Jr. and Sr., Arch. Dermat. and Syph., 1941, 44, 429–440).

**Malachite Green** (CI, 657)—diamond green B, BX or P extra, light green N, new Victoria green extra, O, I or II, solid

green O, Victoria green B or WB—Commission Certified. A feebly basic di-amino tri-phenyl methane dye quite extensively employed as a counterstain for safranin or carmine.

**Malachite Green G,** see **Brilliant Green.**

**Malaria,** see **gametocytes** of.

**Malarial Pigment.** Produced in erythrocytes by action of the parasites, black and distinguishable from carbon by its solubility in concentrated sulphuric acid. Among distinguishing characteristics given by Lison (p. 254) are solubility in dilute alkalis, argentaffine reaction negative, specific stains for lipids negative, likewise reactions for iron. But Morrison and Anderson (D. B. and W. A. D., Public Health Rep., 1942, **57**, 90–94) find that when the pigment within the parasites (Plasmodium Knowlesi) is extracted in such a way as not to influence the spectra of hemoglobin it can be identified spectrophotometrically as ferrihemic acid, or hematin, which does contain iron.

**Mallory's Connective Tissue Stain.** This is name usually given to his anilin blue-acid fuchsin-orange G stain. See also his **Phosphomolybdic** and **Phosphotungstic Acid Hematoxylin Stains.** (Mallory, p. 155). Fix in Zenker's fluid. Imbed in paraffin or celloidin. Remove mercury from sections with iodine or 0.5% sodium hyposulphite. Stain in 0.5% aq. acid fuchsin, 1–5 min. Drain off stain and put in: anilin blue, water soluble, 0.5 gm.; orange G, 2 gm.; 1% aq. phosphotungstic acid, 100 cc., 20 min. or longer. Rinse in 95% alc. 2 or 3 changes until no more stain is removed. Dehydrate in abs. alc., clear in xylol, mount in neutral balsam. For celloidin sections, reduce staining time and pass from 95% alc. to terpineol and mount in balsam. This is one of the most beautiful of all stains and is very widely used. Collagenic fibrils blue, fibroglia, neuroglia and myoglia fibrils red, elastic fibrils pink or yellow. In McClung, p. 405, Mallory and Parker advise 0.25% aq. acid fuchsin and staining in the anilin blue mixture for 1–24 hrs. or for 1 hr. in paraffin oven at 60°C. The modifications of this stain are almost endless.

Adaptation to formalin fixed material is often desirable. Kernohan (J. W., J. Tech. Meth., 1934, **13**, 82–84) has outlined the following method of doing this by mordanting. Wash formalin fixed tissue in running water or in ammonia water for short time. Place in Weigert's primary mordant—potassium bichromate, 5 gm.; chromium fluoride, 2 gm. and aq. dest. 100 cc.—for 4 days and in his secondary mordant—copper acetate, 5 gm.; chromium fluoride, 2.5 gm.; acetic acid (36%), 5 cc.; aq. dest., 100 cc. and formol, 10 cc.—for 2 days. Imbed in paraffin in the usual way.

Rexed, B., and Wohlfart, G., Zeit. wiss. Mikr., 1939, **56**, 212–215 suggest control of pH of the acid fuchsin. It is stated that fresh 0.1% acid fuchsin has pH 4.49 and that increase in alkalinity makes it defective. To prepare one at pH 3.29 ± 0.01, which is recommended, take acid fuchsin 1 gm.; N/10 HCl, 60 cc.; aq. dest. 900 cc.; Störensen's citrate (citric acid crystals, 21 gm.; N/1 NaOH, 200 cc.; + aq. dest. to make 1000 cc.), 40 cc. Most tissues stain in range pH 3–4, red blood cells alone at pH 5–7.

In 1936, Mallory considered (Stain Tech., **11**, 101–102) the most important modifications of his stain to be **Heidenhain's Azocarmine (Azan), the Lee-Brown** and **Masson Trichrome** methods. See **Crossman's** modification and **Pituitary** for special adaptations.

**Mammary Glands.** These can be studied in sections by methods intended to reveal the particular data sought. For general purposes **Hematoxylin and Eosin, Mallory's Connective Tissue Stain,** or **Phloxine-Methylene Blue** is recommended after Zenker fixation. For fat use **Sudan Black** and **Oil Red O** on frozen sections after fixation in 10% formalin or examine in paraffin sections after fixation in Flemming's fluid or some other osmic acid containing mixture.

In the case of the small glands of mice, rats, rabbits and other mammals the method of making whole mounts is invaluable in investigations of the responses of mammary glands to endocrine stimulation. The following is essentially the same technique as that originally described by Turner, C. W. and Gardner, W. U., Agri. Exp. Res. Stat. Bull., Univ. of Mo., 1931, **158**, 1–57: Remove skin and mammary gland. Stretch out and fasten on a cork block with the external surface of the skin down. Fix in Bouin's fluid 24 hrs. Wash in tap water. Dissect away all tissue over the gland which has been tinged light yellow by the picric acid in the fixative. Remove the gland from the skin. Stain in Mayer's **Hemalum.** Wash in 1% aq. potassium alum and then in water. Differentiate in 70% alc. + 2% of hydrochloric acid until the color has been removed from the connective tissue and the acini and ducts of the glands show up in sharp contrast in a light background. Wash in tap water.

Dehydrate in alcohol, clear in xylol, mount in balsam between glass plates and close the edges with sealing wax. Much can be made out when magnified only 2–5 times. Small pieces can be mounted on slides, with edges of cover glasses supported as may be necessary, for examination at higher magnifications. There are many excellent pictures in the paper cited.

For examination of fetal mice, see Turner, C. W. and Gomez, E. T., *ibid*, 1933, **182**, 1–43. Valuable data are given in Turner's chapter on mammary glands in Allen's Sex and Internal Secretions, Baltimore: Williams & Wilkins, 1939, 1346 pp. For techniques to reveal secretory phenomena in mammary glands, see Weatherford, H. L., Am. J. Anat., 1929, **44**, 199–281; Jeffers, K. R., Am. J. Anat., 1935, **56**, 257–277, 279–303. A method for obtaining serial slices of whole human breasts is described by Ingleby, H. and Holly, C., J. Tech. Meth., 1939, **19**, 93–96.

**Manchester Blue** (British Drug Houses Ltd), a dis-azo dye of the benzidine series. In either alcoholic or aqueous solution it gives a sharp deep blue effect (H. G. Cannan, J. Roy. Micr. Soc., 1941, **61**, 88–94).

**Manchester Brown**, see **Bismark Brown Y.**

**Manchester Yellow**, see **Martius Yellow.**

**Mandarin G**, see **Orange II.**

**Manganese.** Histochemical detection uncertain (Lison, p. 98).

**Manganese Dioxide.** Drinker, C. K. and Shaw, L. H., J. Exper. Med., 1921, **33**, 77–98 employed a suspension of fine particles in acacia water to investigate phagocytic power of endothelium because the particles can be seen within the cells and the amounts of manganese in the tissues can be determined by chemical analysis.

**Mann's Fixative** is equal parts 1% aq. osmic acid and sat. corrosive sublimate in physiological salt solution (0.85% NaCl). It is a good way to apply osmic acid for the blackening of fat.

**Mann's Methyl Blue-Eosin Stain.** This is used for protozoa and for inclusions caused by viruses. Sections are deparaffinized, stained 12 hrs. in 1% aq. methyl blue 35 cc., 1% aq. eosin 45 cc. and aq. dest. 100 cc. They are then rinsed in 95% alc., dehydrated cleared and mounted. See **Alzheimer's Modification** of Mann's method.

**Marchi Method.** For degenerating nerve fibers. Modification by Swank, R. L. and Davenport, H. A., Stain Techn., 1935, **10**, 87–90. Details provided by Dr. J. L. O'Leary. Degeneration time of approximately 14 to 20 days. Kill animal by overdose of nembutal or some other barbiturate given intraperitoneally. Open left ventricle, insert cannula into aorta and perfuse with 2.5–5% anhydrous (10% crystalline) magnesium sulfate solution containing 2–3% potassium bichromate. Immediately afterwards remove the brain and spinal cord and put into 10% formalin for 48 hrs. Place slices 3 mm. thick directly, without washing, in: 1% aq. potassium chlorate, 60 cc.; 1% aq. osmic acid, 20 cc.; glacial acetic acid, 1 cc.; 37% formaldehyde (Merck's reagent), 12 cc. Use about 15 volumes of this fluid to 1 of tissue. Agitate and turn over daily. After staining for 7–10 days, wash in running water, 12–24 hrs., dehydrate in 70% and 95% and absolute alcohol and imbed in low viscosity nitrocellulose as described by Davenport, H. A. and Swank, R. L., Stain Tech., 1934, **9**, 134–139. See **Celloidin Imbedding.** Cut 40$\mu$ sections serially, mount on slides, dehydrate to toluol, placing chloroform in absolute alcohol since low viscosity nitrocellulose is soluble in absolute alcohol. Clear in toluol. Mount in clarite X dissolved in toluol. See these authors (Stain Techn., 1935, **10**, 45–52) for artifacts and effects of perfusion in Marchi technique.

**Marchi's Fluid. Müller's Fluid,** 2 parts; 1% osmic acid, 1 part. Fix 5–8 days; wash in running water. Employed to blacken degenerated nerve fibers. See **Nerve Fibers.**

Method, underlying mechanisms involved (Swank, R. L. and Davenport, H. A., Stain Techn., 1934, **9**, 11–19; 1935, **12**, 45–52).

**Marine Blue V**, see **Anilin Blue.**

**Marshall Red** (British Drug Houses Ltd), a disazo dye. Stain sections in sat. aq. solution 20 min. Rinse in aq. dest. Stain in sat. Victoria Green G in 70% alcohol 30 min. Rinse in 95% alcohol, dehydrate, clear and mount in usual way. Myofibrils sage green, nuclei crimson. Advised also for retina (H. G. Cannan, J. Roy. Micr. Soc., 1941, **61**, 88–94).

**Martius Yellow** (CI, 9)—Manchester yellow, naphthol yellow—An acid nitro dye employed by Pianese (G., Beitr. z. Path. Anat. u. Allg. Path., 1896, Suppl. I, 193 pp.) for investigating cancer tissue in association with acid fuchsin. Conn (p. 44) reports good results in staining of plant tissue with CC product.

**Masson's Gelatin Glue.** Method for making sections stick to slides (Masson, P., Am. J. Path., 1928, **4**, 181–212). Dissolve 0.05 gm. sheet gelatin in 20 cc. aq. dest., warming gently. Filter a large drop on each slide on warm plate.

Float paraffin sections on drops. When drops spread place slides upright to drain but do not permit drying. Blot and transfer to dish containing formalin (so arranged that vapor only will act on slides) in oven 45–50°C. For subsequent staining 20 minutes in hot vapor is enough. For silver treatment overnight is suggested.

**Masson's Trichrome.** Stain for connective tissue (Masson, P., Am. J. Path., 1928, **4**, 181–212; J. Tech. Meth., 1929, **12**, 75–90). The following is an abbreviated account of the technique as recommended by Mallory (p. 156). Use 5$\mu$ paraffin sections of Bouin's fluid (3 days) or Regaud's (1 day) fixed tissues. Mordant in 5% aq. ammonio-ferric alum previously warmed to 45–50°C. for 5 min. Wash in water and stain for 5 min. at 45–50°C. in Regaud's hematoxylin (hematoxylin, 1 gm.; 95% alc., 10 cc.; glycerin, 10 cc.; aq. dest., 80 cc.). Rinse in aq. dest. and differentiate in picric alcohol (sat. picric acid in 95% alc., 2 parts; 95% alcohol, 1 part). Wash in running tap water. Stain for 5 min. in: acid fuchsin, 0.3 gm.; Ponceau de xylidine, 0.7 gm.; aq. dest., 100 cc.; glacial acetic acid, 1 cc. Rinse in aq. dest. Differentiate in 1% aq. phosphomolybdic acid, 5 min. Without rinsing add 10 drops sat. aniline blue in 2% acetic acid and leave for 5 min. Rinse in aq. dest. and place again in phosphomolybdic acid. 1% acetic for 5 min. Dehydrate in 95% alcohol, then absolute, xylol and balsam. Collagen, deep blue; neuroglia fibrils, red; nuclei, black; argentaffin granules, black or red. See modifications by Goldner, J., Am. J. Path., 1938, **14**, 237, and Larson, C. P. and Levin, E. J., Arch. Path., 1940, **29**, 272–273.

The difficulty is that the French "*ponceau de xylidine*" cannot be secured. It appears to be similar to ponceau 2R (C.I. 79) but the latter does not give regularly good results. Lillie (R. D., Stain Tech., 1940, **15**, 17–22) suggests the following substitutes for ponceau de xylidine: azofuchsin 3B (C.I., 54), nitrazine yellow and biebrich scarlet (C.I., 280). See the **Biebrich Scarlet** and **Picro-Anilin Blue** method of Lillie.

**Mast Cells,** see **Basophile Leucocytes** and **Tissue Basophiles.**

**Mastoid Process.** Use methods for **Bone.** Technique for measurements of size of air cell system is given by Diamant, M., Acta Radiol., 1940, **31**, 543–548.

**Maximow** (see **Azure II Eosin Hematoxylin** method). He has advised as a fixative 90 cc. Zenker's fluid less acetic acid + 10 cc. formalin. This is essentially **Formalin Zenker.** See **Buzaglo's** connective tissue stain.

**May-Giemsa** stain of Pappenheim (Folia Haematol., Arch., 1917, **22**, 15). This is the same as Jenner-Giemsa. Fix and stain air dried blood smears about 3 min. in May-Grünwald mixture (sat. sol. methylene blue eosinate in methyl alcohol). Add same amount aq. dest. and leave 1 min. Pour off (but do not wash) and add diluted Giemsa's solution. Stain in this 15–30 min. Rinse aq. dest. 1 min. or until desired color is reached. Blot dry. This is a good modification of the ordinary Giemsa's stain because it gives slightly more intense colors.

**May-Grünwald** combined fixative and stain is a sat. sol. of methylene blue eosinate in methyl alcohol (Grübler or Hollborn). If methylene blue eosinate is not available make it as originally described by Jenner (Lancet, 1899, No. 6, 370). Mix equal parts 1.25% water sol. eosin and 1% methylene blue; after 24 hrs. filter; wash ppt. on filter with water; dry and dissolve powder in 200 cc. pure methyl alcohol. It is employed in the May-Giemsa and Kardos-Pappenheim methods for staining blood smears.

**May-Grünwald-Giemsa** stain in one solution. Strumia (M. M., J. Lab. & Clin. Med., 1935-36, **21**, 930-934) gives directions for combining the stains and for use and notes that a standardized product is prepared by Coleman and Bell Co. Intensity of coloration is enhanced by the combination.

**Mayer's Acid Alum Hematoxylin.** The following formula is given by Mallory (p. 73). Dissolve 1 gm. hematoxylin in 1000 cc. aq. dest. with a little heat if required. Add 0.2 gm. sodium iodate and 50 gm. ammonium or potassium alum. When latter is dissolved add 1 gm. citric acid and 50 gm. chloral hydrate. Color turns reddish violet. Does not easily over-ripen.

**Mayer's Acid Carmine.** The Bensleys (p. 131) advise its preparation as follows. Add 4 gm. carmine to 15 cc. aq. dest. + 30 drops hydrochloric acid. Boil until it is dissolved. Add 95 cc. 85% ethyl alcohol. Neutralize with ammonia until the carmine begins to precipitate as seen in a graduate against white paper background. Add 4 more drops ammonia after first precipitation. If this acid carmine stains too quickly, slow it down by dilution with 80–90% alcohol. This gives a fine red nuclear counterstain for tissues vitally stained with **Indigo-Carmine, Trypan Blue** and similar dyes.

**McIlvaine Buffers** after Stitt from Lillie,

R. D., Stain Techn., 1941, **16**, 1–6 who employed them to improve Romanowsky staining after various fixatives. See **Toluidine Blue Phloxinate** Method. (see **Molecular Solution**) To make M/15 citric acid required dissolve 14.01 gm. mono-hydrated crystalline citric acid in 500 cc. aq. dest. and add enough neutral methyl alcohol C.P. to make total volume 1,000 cc. after careful mixing. To make M/15 $Na_2HPO_4$ dissolve 9.47 gm. anhydrous $Na_2HPO_4$ in 500 cc. aq. dest. and make up to 1,000 cc. with methyl alcohol. These, in following proportions listed in cc., give pH values indicated.

| cc. Citric Acid | cc. $Na_2HPO_4$ | pH |
|---|---|---|
| 1.3 | 0.7 | 3.9 (3.873) |
| 1.25 | 0.75 | 4.0 (4.034) |
| 1.2 | 0.8 | 4.2 (4.205) |
| 1.15 | 0.85 | 4.4 (4.44) |
| 1.1 | 0.9 | 4.6 (4.653) |
| 1.05 | 0.95 | 4.8 (4.80) |
| 1.0 | 1.0 | 5.0 (5.042) |
| 0.95 | 1.05 | 5.2 (5.201) |
| 0.9 | 1.1 | 5.4 (5.428) |
| 0.85 | 1.15 | 5.7 (5.696) |
| 0.8 | 1.2 | 5.85 (5.838) |
| 0.75 | 1.25 | 6.05 (6.036) |
| 0.7 | 1.3 | 6.3 (6.29) |
| 0.65 | 1.35 | 6.5 (6.444) |
| 0.6 | 1.4 | 6.5 (6.522) |
| 0.55 | 1.45 | 6.6 (6.60) |

Since it is difficult to measure out these small volumes accurately at least ten times the volume in each case should be taken and the amount not required simply be discarded. For ordinary purposes employ aq. dest. in place of methyl alcohol.

**McJunkin-Haden Buffer** has pH 6.4 and is useful in place of aq. dest. for diluting Giemsa, Wright and other blood stains. Monobasic potassium-phosphate, 6.63 gm.; anhydrous dibasic sodium phosphate, 2.56 gm.; aq. dest., 1000 cc. (Haden, R. L., J. Lab. & Clin. Med., 1923, **9**, 64–65).

**Meckel's Diverticulum.** Literature on (Curd, H. H., Arch. Surg., 1936, **32**, 506–523).

**Megakaryocytes.** These can, like blood cells, be examined in fresh and stained smears of bone marrow. For a determination of their role in platelet formation it is essential to clearly show the granules typical of both. This can best be done in sections of bone marrow prepared by:

1. Wright's method (Wright, J. H., J. Morph., 1910, **21**, 263–277). After fixation in sat. mercuric chloride in 0.9% aq. NaCl, dehydrate in alcohol, follow with acetone, clear first in thick cedar oil and then in xylol, embed in paraffin. Sections deparaffinized are covered with equal parts stain (polychrome methylene blue solution 3 parts and 0.2% eosin yellowish in methyl alcohol 10 parts) 10 min. A metallic looking scum forms but the stain should not be allowed to precipitate. Stop staining when cytoplasm looks bright red and reticular fibers light red. Wash in water, dehydrate in acetone, clear in turpentine and mount in thick colophonium in pure turpentine oil. See Wright's colored plates. In place of the fixative suggested, Downey (Folia haematol., Archiv, 1913, **15**, 25) uses commercial formalin 10 cc. and sat. mercuric chloride in 0.9% aq. NaCl 90 cc.

2. Kingsley's method (Kingsley, D. M., Folia Haemat., 1937, **57**, 87–98). Fix in Downey's fluid (given above) 4 parts, saturated picric acid 1 part, 24 hrs. Wash in running water, 18–24 hrs. Dehydrate through alcohols up to 70%, ½–1 hr. each. 80% alc. + iodine, overnight. 95% alc., 45 min. Repeat with fresh alc. *N* butyl alcohol (technical), 1 hr. Repeat with fresh. Paraffin (58°C.), ½ hr., then 3 more changes, each ½ hr. Imbed. Prepare stock solutions A: methylene blue (U.S.P. med. 88%), 0.065 gm.; methylene azure A (80%), 0.01 gm.; glycerin, C.P., 5 cc.; $CH_3OH$ (C.P.), 5 cc.; aq. dest., 25 cc.; buffer (pH, 6.9), 15 cc. B: methylene violet (Beruthsen 85%), 0.013 gm.; eosin, yel. (92%), 0.45 gm.; glycerine, 5 cc.; $CH_3OH$, 10 cc.; acetone, C.P., 35 cc. The buffer is 40 cc. of A = 9.078 gm. $KH_2PO_4$ per liter + 60 cc. of B = 11.876 gm. $Na_2HPO_4 \cdot 2H_2O$ per liter of aq. dest. Immediately before use mix equal parts of stock stains A and B. After washing deparaffinized sections in aq. dest. stain 8–10 min. Wash off in current of aq. dest. Wash in aq. dest. 100 cc. + 1% acetic acid, 0.8 cc. Wash again in aq. dest. to remove acid. Blot. Rinse in acetone, 100 cc. + 0.001 gm. eosin + 4 cc. 1% acetic acid. Rinse in *n* butyl alc. + a little eosin. Neutral xylol several changes. Mount in neutral xylol dammar. See Kingsley's plate for colors. Granules dark red. It is important to fix the bone marrow promptly after death or to obtain it by biopsy.

**Megaloblasts,** see **Erythrocytes,** developmental series.

**Meibomian Glands.** Whole mounts of the glands stained with Sudan IV in a transparent background by a method described for **Sebaceous Glands.**

**Meissner's Corpuscles.** To investigate by supravital staining with methylene blue in skin of amputated fingers, see Weddell, G., J. Anat., 1940–41, **75,**

441–446. Skin from general body surface will not do because of rarety of the corpuscles.

**Meissner's Plexus,** see **Auerbach's.**

**Melanins.** Lison (p. 248) gives many differential microchemical properties from which the following are selected. Extreme resistance to most chemicals, not modified by concentrated acids but soluble in concentrated alkalis. They are depigmented by oxydants. Thus, Schultze treats them with diaphanol (chlordioxyacetic acid) for 24 hrs. in hermetically sealed container in darkness; and Alfiere treats sections with 0.1% potassium permanganate 2–24 hrs.; washes with much water, treats with 0.3% oxalic acid and again washes. Their power of reducing ammoniacal silver nitrate, Lison regards as very characteristic. Melanins occur normally in epidermis, hair, choroid of eyes. Greatly increased in Addison's disease. Contain no iron or fat. A method for the collection of melanin for analysis by differential **Centrifugation** is described by Claude, A., Trans. New York Acad. Sci., 1942, II, 4, 79–83. See **Dopa Reaction** for melanogen in melanoblasts.

**Melanoblasts,** see **Dopa Reaction.**

**Meldola's Blue,** see **Naphthol Blue R.**

**Mercuric Chloride** (corrosive sublimate) in various combinations is an excellent fixative. It can be used in saturated aq. sol. plus 5% acetic acid or in saturated alc. sol. with the same amount of acetic acid. See (1) with formalin, glacial acetic and physiological saline for **Centrosomes,** (2) sat. in 0.9% aq. sodium chloride for **Megakaryocytes,** (3) sat. in 70% alcohol + 5% acetic for **Mitosis,** (4) sat. aq. + equal parts 2.5% aq. potassium bichromate for **Neutral Gentian,** (5) sat. aq. with equal parts abs. alcohol for **Thymonucleic Acid,** and (6) with nitric acid for **Urea.** The mercuric chloride is removed from the sections by Lugol's iodine solution. See also fixatives of **Zenker, Gilson, Rabl** and **Petrunkewitsch.** Zinc chloride is suggested as substitute for mercuric chloride in Zenker's fluid (Russell, W. O., J. Techn. Methods & Bull. Int. Asso. Med. Museums, 1941, 21, 47).

**Mercurochrome 220.** Trade name for dibrom-oxy-mercuri-fluorescein. Can be used as substitute for eosin (Baldwin, W. M., Anat. Rec., 1928, 39, 229) but it has little to commend it.

**Mercury,** microchemical tests for.

1. Method of Almkvist-Christeller. Fix tissues 2 days in sat. aq. picric acid, 100 cc.; 25% nitric acid 1 cc., saturated with $H_2S$ gas, filtered after 1 day. After fixation wash in running water for 24 hrs. Imbed in paraffin. Mercury appears as black ppt. of sulphide. Lison (p. 102) explains that it is necessary to make parallel tests for iron because this method changes iron into the black sulphide which could be mistaken for the sulphide of mercury. Simonet (M., Arch. d'Anat. Micr., 1929, 25, 372–381) uses instead fixation for 10 hrs. in equal parts alcohol and chloroform, 100 cc., + nitric acid, 2 cc. the mixture saturated with $H_2S$ by bubbling.

2. Method of Brandino (G., Studi Sassari, 1927, 5, 85). Fix in formalin or in alcohol. Treatment of sections with 1% sol. of diphenylcarbazide which forms with mercury a violet ppt. Gives results with organs of persons killed by mercury poisoning kept in formalin 17 years (Lison, p. 102).

Intravenous injections of colloidal solutions of mercury in rabbits are described by Duhamel, B. G., C. rend. Soc. de Biol., 1919, 82, 724–726.

**Mesentery** spreads, sections and cultures. Maximow, A., Arch. f. exper. Zellf., 1927, 4, 1–42 (nice colored plates).

**Metachromasia,** see **Metachromatism.**

**Metachromatism** (metachromasia) is the property of certain dyes to stain (G., meta, beyond) the usual color (G. chroma). The action of some impure methylene blues is sometimes cited as an example. Thus polychrome (many colored) methylene blue stains some objects blue and certain granules reddish. This methylene blue is however a mixture of methylene blue and methylene red. The latter dye accounts for the staining beyond. Orcein colors nuclei blue and cytoplasm pink. Safranin stains nuclei in its ordinary solution color (red) and the ground substance of cartilage that of its free color base (orange). Michaelis (Lee, p. 136) thinks that the appearance of the color base is not occasioned by the alkalinity of the objects stained. The red stain of mucin by thionin can be altered to blue by alcohol and be shifted back to red by water. For colored plates showing metachromatic staining of mast cells, see Maximow, A., Arch. f. mikr. Anat., 1913, 83 (1), 247–289. Metachromasia of acid dyes is increased by adding strychnine, quinine or clupein and of basic dyes by gum arabic or other negatively charged colloid (Bank, O. and Hungenberg de Jona, H. G., Protoplasma, 1939, 32, 489–516).

**Metamyelocytes,** see **Leucocytes,** developmental series.

**Metanil Yellow** (CI, 138)—acid yellow R, orange MNO or MN, soluble yellow OL, tropaeolin G, yellow M—An acid monoazo dye employed in the Masson tech-

nique, see Foot, N. C., Stain Techn., 1933, 8, 101–110.

**Methyl Alcohol,** see **Elementary Bodies.** It is much used in many techniques.

**Methyl Benzoate.** Refractive index close to that of cedar wood oil. It can be used in place of immersion oil. In addition it is a substitute for absolute alcohol and an excellent clearing agent but it is expensive. See **Ceresin** imbedding.

**Methyl Blue** (CI, 706)—cotton blue, Helvetia blue—Widely used. See **Mann's Methyl-Blue Eosin** and staining of **Elementary Bodies.**

**Methyl Blue-Eosin,** see **Mann's.**

**Methyl Eosin** (CI, 769). The methyl ester of eosin Y, see **Eosins,** choice of.

**Methyl Green** (CI, 684)—double green, light green—This basic triphenyl methane dye is crystal violet (hexa-methyl pararosanilin) into which a seventh methyl group has been incorporated. Conn (p. 130) points out that this is loosely bound so that some methyl or crystal violet is always present with the methyl green to which circumstance the metachromatic properties of the dye are partly due. Methyl green is not as stable as most dyes and cannot therefore be kept too long in the powdered state. It is very similar to **Ethyl Green.**

**Methyl Green-Pyronin** (Pappenheim). Sections of formalin-Zenker fixed tissues are stained about 6 min. in: methyl green 0.5 gm.; pyronin Y, 0.5 gm.; alc. 2.5 cc.; glycerin 20 cc.; aq. dest. 0.5% carbolized 100 cc. Rinse in aq. dest.; dehydrate in acetone; clear in cedar oil followed by xylol and mount. Optimum time of staining must be determined experimentally. A brilliant stain particularly for lymphocytes and plasma cells. Very useful for spleen and lymph nodes. (Slider and Downey in McClung's Microscopical Technique, p. 342).

**Methyl Orange** (CI, 142)—gold orange MP, helianthin, orange III, tropaeolin D—A slightly acid mono-azo dye widely employed as an **Indicator.**

**Methyl Red** (CI, 211). A slightly acid mono-azo dye widely used as an **Indicator.** See also Carter, J. S., J. Exp. Zool., 1933, 65, 159–179 for vital staining of rabdites of **Stenostomum** with methyl red.

**Methyl Salicylate** (oil of Wintergreen) is employed in **Spalteholz Method** of clearing.

**Methyl Violet** (CI, 680)—dahlia B, gentian violet, Paris violet, pyoktaninum coeruleum—Exists in various shades 2R, R, B, 2B, 3B, etc., depending upon proportions of the mixture of tetra-, penta- and hexa-methyl rosanilins. R indicates reddish and B bluish. 2B is the one which Conn (p. 123) regards as most satisfactory whenever methyl violet, or one of the redder types of gentian violet, is requested. (It is Commission Certified.) The pure hexamethyl compound is called crystal violet—a dye much in demand.

**Methylene Azure** (CI, 923). A basic thiazin dye long recognized as a component of **Polychrome Methylene Blue.** Conn (p. 76) says that the term, methylene azure, should be discarded because it is composed of three components **Azure A, B,** and **C** which see.

**Methylene Blue** (CI, 922)—Swiss blue—Conn (p. 80) says that this basic thiazin dye is theoretically tetra-methyl thionin but the homologues of lower methylation are practically always present; he lists the following grades: methylene blue BX, B, BG, BB, and methylene blue chloride. The last named is Commission Certified and least toxic. Methylene blue Med. U.S.P. is required to be zinc free and is also satisfactory. New methylene blue N (methylene blue NN) is a basic dye of the same type but of a slightly greener shade. Conn (McClung, p. 595) states that it was apparently in certain lots of prewar methylene blue. Methylene blue O is the same as toluidin blue O which resembles azure A, a component of methylene azure produced by polychromizing methylene blue. Another of the series is methylene blue GG but it has no particular advantage. Probably no dye, other than hematoxylin and eosin, is more widely used. The oxidation products of methylene blue are described by Holmes, W. C., Stain Techn., 1926, **1,** 17–26 and the influence of pH on staining of plasma cells and lymphocytes by Kindred, J. E., Stain Techn., 1935, **10,** 7–20. Its cytological action has been fully studied by Ludford, R. J., Arch. f. exp. Zellf., 1935, **17,** 339–359. It is an excellent counterstain for **Acid Fast Bacilli.** See **Polychrome Methylene Blue, Loeffler's Alkaline Methylene Blue, Nerve Endings, Phloxine Methylene Blue, MacNeal's Tetrachrome, Pancreas, Protozoa,** etc.

**Methylene Blue NN,** see **New Methylene Blue N.**

**Methylene Blue T 50** or **T Extra,** see **Toluidin Blue O.**

**Methylene Blue Eosinate,** see **May-Grünwald** fixative and stain.

**Methylene Green** (CI, 924). This basic thiazin dye is mono-nitro methylene blue. Conn (p. 86) says that it is occasionally employed as a substitute for

methyl green and gives good results as counterstain for eosin.

**Methylene Violet.** Commission Certified. This feebly basic thiazin dye is, as Conn (p. 86) explains, formed whenever methylene blue is heated with a fixed alkali or alkali carbonate. It may be purified by recrystallization but little is to be gained. The dye is not much used.

**Michrochemical Reactions.** For the microscopic identification of particular elements or substances some microchemical reactions are available but it is difficult to sharply distinguish them from other techniques not usually styled microchemical. An attempt is made to list them under the objects demonstrated: **Lead, Iron, Vitamin C, Peroxidase,** etc. Many are generally known under personal names. See for example: **Axenfeld** (proteins), **Burchardt** (gold), **Carr-Price** (vitamin A), **Feulgen** (thymonucleic acid), **Gmelin** (bile pigments), **Lilienfeld-Monti** (phosphorus), **Millon** (tyrosin), **Romieu** (proteins), **Schiff** (aldehydes), **Vulpian** (epinephrine), etc.

**Microdissection.** In the selection of this method for use in any particular problem it is well to bear in mind several considerations. It is of particular value in the direct examination of large cells easily isolated, like sea urchin eggs, and of tissues that exist in thin sheets, like highly vascularized membranes which can be easily approached in the living state without serious injury. The data to be secured relate chiefly to the responses of the cells to the mechanical stimulus of the microneedle, to the character of the connections between fibers, cells and parts of cells as determined by their resistance to attempts to separate them and to the physical consistency of cellular and nuclear membranes and of cytoplasm and nucleoplasm. Moreover individual cells can be isolated by microdissection just as Barber was able to isolate single bacteria by the pipette which he introduced and which was in fact the inspiration of G. L. Kite's first microdissection apparatus. Today this has been very greatly improved chiefly by Chambers and Peterfi. An excellent account of the apparatus required and of its proper use is provided by Robert Chambers and M. J. Kopac in McClung, pp. 62–109, and more recently by Chambers in J. Roy. Micr. Soc., 1940, **60**, 113–127. However an attempt should not be made to learn the technique *de novo* from the printed word. Actual experience under the supervision of a master will save valuable time. A helpful preliminary is to view motion picture films of microdissections which can be obtained on loan from the Wistar Institute of Anatomy in Philadelphia. See Colloquium on Micrurgy (Microdissection), edited by J. C. Regniers, Publisher: Charles C. Thomas, In Press.

**Microglia.** Method for impregnating with silver in pyroxylin (celloidin) sections (Weil, H. and Davenport, H. A., Trans. Chicago Path. Soc., 1933, **14**, 95–96). Wash 15$\mu$ sections in aq. dest. Treat for 15–20 sec. with silver solution (made by adding 10% aq. silver nitrate drop by drop from a burette to 2 cc. conc. ammonia (28%) shaking to prevent ppt. formation until about 18 cc. have been added and the solution has become slightly opalescent). Transfer to 15% formalin, moving section rapidly until coffee-brown in color. Pass through 3 changes aq. dest. Dehydrate in alcohol, clear in xylol and mount in balsam.

**Microglia and Oligodendroglia.** In frozen sections 20–40$\mu$ of formalin fixed material. Immediately place them in aq. dest. + 20 drops ammonia per 100 cc. Thence pass directly to 5% aq. ammonium bromide 40–50°C. 10–15 min. Equal parts ammonia, pyridine and aq. dest. 2 min. Then 3–5% aq. sodium sulfite, 2–3 min. Pass through and shake in 3 changes 1 min. each of following: 8 parts 5% aq. sodium carbonate, 2 parts, 10% aq. silver nitrate + ammonia till ppt. Reduce in 1% formalin less than 1 min. Wash, dehydrate clear and mount (King, L. S., Arch. Neurol. and Psychiat., 1937, 38, 362–364).

**Microincineration.** By this method the mineral constituents of cells and intercellular materials, which are not volatile at the high temperature employed, can be examined microscopically in very nearly if not quite the position occupied by them *in vivo*. Because most of these minerals cannot be visualized in routine histological preparations and because the technique can be applied in the study of all parts of the body in pathological as well as in normal conditions the horizon has been greatly extended for histophysiologists. A. Policard deserves credit for its first use abroad and G. H. Scott for its improvement and introduction into this country. A detailed exposition of apparatus, technique and applicability of findings in many problems is given by Scott in McClung, pp. 643–665. The latest types of incinerators in which temperature and time are closely controlled cannot be purchased on the market but can be made on specifications to be obtained from Dr. G. H. Scott, Dept. of Anatomy,

University of Southern California Medical School, Los Angeles.

The technique requires care but is not particularly difficult. Before incineration the tissues are fixed in a fluid free from minerals, such as 10% formalin in absolute alcohol. After paraffin imbedding the sections are incinerated and the distribution of the mineral residue is examined by reflected light in the dark field. This is the simple method which has been much used to advantage. It is however open to the criticism that some of the mineral salts may shift their position during the preparation of tissues for microincineration. This can be partly or altogether obviated by the **Altmann-Gersh** freezing and drying technique.

Qualitative study of the mineral residue is somewhat limited. The dense white ash observed in the dark field is chiefly *calcium* but *magnesium* gives a similar ash and satisfactory methods for demonstration of the relative amounts of these two elements in any particular white ash remain to be discovered. It is generally assumed but it has not been proved that a finely divided faintly bluish white ash is that of *sodium* and *potassium*. But *silicon* can be recognized with assurance because it retains its crystalline structure and is therefore doubly refractile when viewed in polarized light. Fortunately *iron* can easily be detected by the red color of its oxide. The special methods advocated for *lead* and *uranium* are outlined by Scott in McClung.

On the quantitative side relative differences in amount of residue can be determined fairly accurately by careful study in the dark field. Scott has developed a method for the photoelectric measurement of relative amounts in different parts of the same section but the absolute amounts cannot be expressed in grams per cc. of tissue. See **Electron Microscope, Histospectrography** and **Ultraviolet Photomicrography.**

**Microinjection.** This is an important extension of microdissection whereby various fluids are injected directly into the cytoplasm or nuclei of living cells. It is capable of yielding information on **Permeability, Hydrogen Ion Concentration, Oxidation-Reduction Potential** which cannot be secured in any other way, but in reaching conclusions due allowance must be made for the fact that cells thus treated are of necessity severely injured. Microinjection with glass pipettes but without an expensive micromanipulator can yield worthwhile results as described by Knower (McClung, pp. 51–61) but for direct work on cells the micromanipulator is essential.

**Micrometry** is the measurement of an object observed microscopically. This can be done either by using an ocular micrometer in which there are lines which can be accurately moved the length of the structure to be measured or by inserting a ruled disc in an ordinary ocular with which it can be compared. Both must be standardized in relation to a micrometer slide generally ruled with lines $10\mu$ apart.

**Micromicron** ($\mu\mu$) = 1/1,000,000th part of a micron = 1/1,000,000,000th part of a mm. = $10^{-9}$ mm. = 0.000,001 micron = $10^{-2}$Å. Unfortunately often used synonymously with millimicron ($m\mu$) = 0.001 micron = 10Å.

**Micron** (Gr. *Mikros*, small) expressed by Gr. letter $\mu$ = approximately 1/25,000 inch = 1/1000 part of a mm. = 0.001 mm. = $10^{-3}$ mm. = 10,000 Å (see **Millimicron** and **Micromicron**).

**Microphotometer,** see **Photoelectric.**

**Microscope.** The ordinary microscope usually has darkfield equipment and needs no description. A special illuminator to throw light down on the object has been devised (Preston, J. M., J. Roy. Micr. Soc., 1931, **51**, 115–118). **Centrifuge, Fluorescence, Electron, Polarizing** and **Darkfield Microscopes.**

**Microtome Knife,** sharpening. There is no easy method. Care and long practice are essential. (See Bensleys, p. 57.) For the usual oil and water stones a ground glass is now sometimes substituted (Uber, F. M., Stain Techn., 1936, **11**, 93–98).

**Micrurgical Technique** (Gr. *micros*, small + *ergon*, work) is referred to under the heading of microdissection.

**Milk,** bacteria in, a modification of Newman technic (Broadhurst, J. and Paley, C., J. Am. Vet. Med. Assoc., 1939, **94**, 525–526). To prepare stain add 0.4 cc. conc. $H_2SO_4$ to 54 cc. 95% alcohol. Mix with 40 cc. technical tetrachlorethane in flask and heat to 55°C. but no higher. Add about 1.0–1.2 gm. methylene blue while mixture is still hot. Shake until dye goes into solution. Then add 8.0 cc. 1% basic fuchsin in 95% alcohol. Mix, cool, filter and put up in glass stoppered bottle. Spread 0.01 cc. milk over area of 1–2 sq. cm. on slide. Dry on flat warm surface 5 min. Flood with stain 15 sec. Drain off excess and dry while flat with gentle heat. Wash in cold water till all blue is removed and a faint pink color appears. Dry and examine.

**Millimicron** ($m\mu$) = 1/1000th part of a micron = 1/1,000,000th part of a mm. =

$10^{-6}$ mm. = 0.001 micron = 10 Å (see **Micromicron**).

**Millon's Reaction.** For microchemical purposes it is necessary, as Bensley and Gersh (R. R., and I., Anat. Rec., 1933, 57, 217–233) point out, for the reagent to act without the aid of heat, to give almost immediately with tyrosin *in vitro* an intense red color yielding red ppt. not changing to yellow within 24 hrs. They give the following directions. Add 600 cc. aq. dest. to 400 cc. conc. nitric acid (sp. gr. 1.42) making 40% by volume. After 48 hrs. add 1 part to 9 parts aq. dest. Saturate with mercuric nitrate crystals frequently shaking several days. To make the reagent take 400 cc. filtrate, add 3 cc. original 40% solution plus 1.4 gm. sodium nitrite. Mount sections (preferably after freezing and drying technique) to slides without using water. Immerse in reagent in cold. Maximum reaction should be within 3 hrs. when sections show noticeable rose color. However use several slides, remove them from reagent in a Coplin jar at intervals, dip immediately in 1% aq. nitric acid, dehydrate quickly in absolute alcohol, clear in xylol and mount in balsam. Bensley and Gersh found that mitochondria are positive to Million's reagent.

**Mineral Oil,** reactions in tissue to fat stains after various fixations (Black, C. E., J. Lab. & Clin. Med., 1937–38, **23,** 1027–1036).

**Mingazzini Phenomenon** in intestinal villi interpreted as an agonal or early postmortem change (by Macklin, C. C. and M. T., J. Anat., 1926, **61,** 144–150).

**Mites.** The techniques given for **Ticks** and **Insects** are applicable for making whole mounts. The simple creosote method (see **Insects**) is recommended.

**Mitochondria** (G. *mitos*, thread + *chondros*, grain). Granules, rods and filaments existing in the cytoplasm of practically all living cells of plants and animals. They can be studied in living cells unstained, after supravital staining and in fixed tissues.

In mammals the best place to observe them *unstained* is in pieces of pancreas cut so small that when mounted in a little physiological salt solution they are flattened out by the pressure of the cover glass. The distal poles of the acinous cells, facing the glandular lumen, may be identified by densely packed, highly refractile zymogen granules. The proximal poles are nearer the surrounding blood vessels and comparatively free from zymogen granules. In them careful search, with the aid of a good oil immersion objective, will reveal the mitochondria as delicate but slightly refractile filaments oriented in general with their length parallel to the length of the acinous cell. Even when well flattened such preparations are too thick for satisfactory examination in the dark field. When, however, a mount of fresh blood is studied in dark field the mitochondria can be distinguished as brilliantly illuminated short rods and granules in the lymphocytes in which they are not obscured, as in the granular leucocytes, by masses of specific granules. Beautiful illustrations of mitochondria seen in the dark field are provided (Strangeways, T. S. P. and Canti, R. G., Quart. J. Micr. Sci., 1927, **71,** 1.)

The easiest way to demonstrate mitochondria *supravitally stained* is to place on each of a series of say 6 slides a small drop of 1:10,000 janus green B (diethylsafraninazo dimethylanilin chloride) in 0.85% sodium chloride solution. The dye should be added from a 1% stock solution in distilled water because the powder does not dissolve easily in salt solution. Prick a finger and touch a small amount of blood to each lot of janus green and cover each immediately. Do not wait to cover until blood has been added to all of them. The weight of the cover glass is sufficient to spread the mixture. If the right amounts of stain and blood have been used the cover glass will settle down on a very thin film of fluid. If too much of either has been used it will float on the fluid and it will not be possible to see clearly. After about 5 or 10 minutes the mitochondria will be seen colored deep bluish green, first in the lymphocytes and later among the granules in the other white cells. To study the preparations at leisure it may be desirable to prevent evaporation by ringing with warm vaseline. For colored illustrations see Cowdry, E. V., Internat. Monatschr. f. Anat. u. Physiol., 1912, 29, 1–31.

Another satisfactory method is to supravitally stain the mitochondria in the pancreas by vascular injection as described originally by Bensley, R. R., Am. J. Anat., 1911, 12, 297–388. About 1 liter of solution is put in a bottle, from the bottom of which a glass tube leads off, or from which the fluid is syphoned through a bent glass tube. About 6 feet of rubber tubing connect this with a glass cannula. The rubber tube is supplied with a clamp. Artery forceps do nicely. A guinea pig, or other animal of suitable size, is killed and bled from the throat because removal of a good deal of the blood facilitates the injection. The cannula is inserted into the aorta through the left ventricle, or into the

thoracic aorta directly, and tied in place. In the former case the branches going up toward the head and arms must be ligated. When all is ready hoist the injection bottle about 4 or 5 feet above the animal and remove the clamp. Open the right auricle so that blood and solution can flow out. In about a minute open the abdomen by a long medial incision but do not display the pancreas. To make sure that all the vessels in the pancreas are being perfused by the solution it is desirable to momentarily clamp the superior vena cava and thus let the solution back up a little under pressure. Now lay bare the pancreas. When the optimum staining is obtained, usually about 10 minutes after the beginning of the injection, it should be slightly swollen, owing to separation of lobes and lobules by increase in tissue fluid, and of a uniform fairly dark bluish green color. Remove the pancreas and place it in salt solution. For examination it is essential to take very small pieces not more than 1 mm. in diameter. Mount them in a little salt solution on slides so that they will flatten by the pressure of the cover glasses, one piece per slide. Study at low magnification shows irregular masses of small deeply stained cells. These are the islands of Langerhans. It is in the acinous tissue, which is less deeply colored, that search should be made for the mitochondria. Identify first the distal poles of the cells charged with zyomgen granules. Then look for greenish blue stained mitochondria in the proximal parts of the cells. After a time the oxygen in the center of the tissue is used up, the dye becomes bleached to a leucobase and then to a pink colored base (diethylsafranin). This method of supravital staining of mitochondria with janus green can be used for any tissue in the body. It is particularly recommended for the pancreas because its lobules are thin, and easily separated without mechanical injury.

Other supravital stains for mitochondria are **Diethylsafranin, Janus Blue, Janus Black 1, Pinacyanol** and **Rhodamin B,** which see. When a very dilute solution of methylene blue is applied to mitochondria in tissue cultures they can be stained a brilliant blue (Ludford, R. J., Arch. f. exp. Zellf., 1935, 17, 339-359). It is not unlikely that, in conditions difficult to define, a considerable number of dyes will color mitochondria supravitally. When *fixed tissues* are to be used the choice of method is important. The difficulty with the osmic acid containing fixatives is that they penetrate poorly. The best fixative is the formalin bichromate fluid of **Regaud** followed by mordanting with 3% potassium bichromate and the best stain is probably **Anilin-Fuchsin Methyl Green** as used by Bensley. See methods of **Altmann, Benda, Champy-Kull, Regaud** and **Volkonsky,** and McClung (pp. 265–274). Mitochondria can now be collected by **Centrifugation** and subjected to direct chemical analysis (Bensley, R. R. and Hoerr, N. L., Anat. Rec., 1934, **60,** 251–266; 449–455).

**Mitochondria** and **Bacteria.** Demonstration in the same cells. See Cowdry, E. V. and Olitsky, P. K., J. Exper. Med., 1922, **36,** 521–533, Cowdry, E. V., Am. J. Anat., 1923, **31,** 339–343. Stain as for mitochondria with **Anilin Fuchsin** and **Methyl Green.** Mitochondria are colored crimson. When the bacilli are acid fast as in leprosy they are colored a dark reddish purple; but when they are not acid resistant they are stained bluish green.

**Mitosis** (G. *Mitos*, thread). Indirect nuclear division in which the chromatin forms a thread which breaks up into chromosomes.

Material should be freshly fixed, less than half hour after removal. But mitosis can be seen in some tissues 24 hrs. or longer after death, especially if the body is kept at a low temperature but the number is less and the details not so clear as after quick fixation (Mallory, p. 108). Sat. mercuric chloride in 70% alc. plus 5% acetic acid, Zenker's fluid, formalin-Zenker, Bouin's fluid and Flemming's strong fluid are satisfactory fixatives but the last named penetrates very badly.

The most beautiful stain for mitotic figures is safranin light green but the mitoses can be more clearly distinguished without the green counterstain. Simply deparaffinise and stain sections in anilin-safranin (Babes), wash quickly in tap water, differentiate in acid alcohol until the resting nuclei are less intensely colored than the dividing ones, wash in 95%, dehydrate in abs. clear in xylol and mount in balsam.

Another excellent method is to apply the Feulgen reaction for **Thymonucleic Acid** to sections of tissues preferably fixed in Carnoy's fluid or acetic sublimate. This demonstrates thymonucleic acid in the chromatin, and the dividing nuclei, as with safranin, are more deeply stained than the others. This method is displacing the older safranin technique.

To demonstrate mitosis in whole mounts of epidermis place freshly excised skin (circumcision specimen pre-

ferred) in 0.1% aq. acetic acid in the icebox over night. Wash quickly in aq. dest. Strip off the epidermis with needles, stain it like a section with anilin-safranin or with Harris' hematoxylin and mount with the outer surface uppermost. This technique could probably be adapted to relatively flat epithelia of the respiratory digestive, urinary and genital systems.

In order to reveal the maximum number of mitotic figures it is important to study the mitotic rhythm of the particular tissue or organ and take tissues at the peak which in the case of the human foreskin is probably between 9 p.m. and midnight (Cooper, Z. K. and Schiff, A., Proc. Soc. Exp. Biol. & Med., 1938, **39**, 323–324).

To experimentally increase the number of mitosis use colchicine which arrests the process chiefly in the metaphase by causing failure of the mitotic spindle to form and function (Ludford, R. J., Arch. f. exper. Zellf., 1936, **18**, 411–441). Consequently as long as the cells are under the influence of colchicine—a matter of a few hours only—mitosis begins as usual; but, since it is not completed, the proportion of mitotic figures to resting nuclei is temporarily greatly increased. Sodium cacodylate, auramine and other substances listed by Ludford likewise influence mitosis. For checks on the method of estimating growth by counting arrested mitoses, see Paletta and Cowdry (F. X. and E. V., Am. J. Path., 1942, **18**, 291–311). Aisenberg (E. J., Bull. d'Hist. Appl., 1935, **12**, 100–122) has found that mitosis of epidermal cells is arrested in the metaphase simply by passing a ligature around a frog's leg and keeping the foot in distilled water. The mitoses accumulate in large numbers but continue when released from the hypotonic environment. Aisenberg (*ibid.* 1936, **13**, 265–286) also discovered low concentration of ethyl alcohol to stimulate mitosis, 0.4–0.8 M to arrest in metaphase, 1.2–1.5 M. to cause gelatinization of mitosis and higher concentrations to kill the cells.

**Molecular Film Technique,** see Taylor, H. S., Lawrence, E. O., and Langmuir, I., Molecular Films, the Cyclotron and the New Biology, Rutger's University Press, 1942, 95 pp.

**Molecular Solution** is the molecular weight of the substance in grams made up to 1 liter with aq. dest. Thus *M* oxalic acid $(COOH)_2 \cdot 2H_2O$ is 126 gms. with aq. dest. added to 1 liter; but *N* oxalic acid is half of this concentration. See **Normal Solutions.**

The molecular weight expressed in grams is called the gram-molecular weight or mole.

Millimole is 1/1000 of a mole.

Milligram equivalent (milliequivalent). The equivalent weight, the gram-equivalent, or the equivalent of a substance is the weight in grams which in its reaction corresponds to a gram atom of hydrogen, or of hydroxyl, or half a gram atom of oxygen, or gram atom of a univalent ion. Milliequivalent is 1/1000 of the equivalent weight, i.e., the equivalent weight of sodium carbonate is $\frac{1}{2}$ the molecular weight, or 53.0. Therefore, the milliequivalent (m.e.) or the weight in 1 ml. of normal solution is 0.0530 gm.

**Mono-Azo Dyes.** Amarnth, azo fuchsin, benzene-azo-α-naphthylamine, bordeaux red, brilliant yellow S, chromotrope 2R, chrysoidin Y, fast yellow, janus green B, metanil yellow, methyl orange, methyl red, narcein, nitrazine, oil red O, orange G, orange I, orange II, orange IV, ponceau 2R, sudan R, thiazine red R.

**Monocytes.** When "typical" these are easily recognized in stained blood smears and in supravital preparations but there is no technique by which they may always be distinguished from all **Lymphocytes** and **Macrophages.** That is, they possess no single feature, like the eosinophile granules of eosinophile leucocytes, for their certain identification (see Cowdry, p. 66–71). They ingest particulate matter including **Trypan Blue** and similar vital stains and are therefore to be considered as components of the **Reticulo-Endothelial System.** Many of their properties can to great advantage be investigated in **Tissue Cultures.** The best way to demonstrate the remarkably close relation that may exist between monocytes and contained bacilli is to stain leprous tissue for acid fast bacilli (see **Leprosy Bacilli**). See **Bacterium Monocytogenes.**

**Monolayer** technique is a physico-chemical line of investigation that gives valuable data on the structure of protein and lipoprotein films and consequently on the plasma membrane of cells. *See* Schulmann (Bourne, pp. 51–67).

**Mordant** (L. *mordere*, to bite), a substance, like alum, employed to make a dye bite into the tissue and hold on. The dye combines with the mordant which is itself in high concentration in the structures to be stained. In the **Iron Hematoxylin** technique the sections are mordanted with iron alum. They are briefly washed in aq. dest. to remove some of the excess mordant. Then they are stained with a dilute aqueous solu-

tion of hematoxylin and differentiated in the mordant which draws out most of the hematoxylin until it remains only in the structures which took up the mordant most energetically in the first place and which therefore alone remain colored. Copper salts are also good mordants. See **Weigert's** mordants.

**Motor End Plates.** The particular morphological type of nerve ending in muscle does not concern us here; but reference can be made if desired to the classification by Hines, M., Am. J. Anat., 1931, 47, 1–55. The methods advocated for histological demonstration are legion. Reference is made to 2 gold techniques (**Graven's** and **Carey's**) and to 1 silver method (**Chor's**). The former can be ultimately traced back to Ranvier and the latter to Cajal. See also techniques described under **Nerve Endings.**

**Mosquito** larvae, technique of raising anopheline (Bates, M., Am. J. Trop. Med., 1941, 21, 103–122).

**Mounting Media.** The refractive index of the medium is important and a table giving the indices for many substances used is supplied by Lee (p. 218). As pointed out, the greatest transparency is secured when the refractive indices of media and tissues are equal and media of lower index than the tissues give somewhat greater visibility of tissue components, while those of higher index provide less visibility. There are many media to choose from, the refractive indices of which are more or less satisfactory. The selection will depend more upon whether the medium can be employed for the particular tissue and its relative permanence.

For frozen sections and tissues to be mounted from water and aqueous solutions various glycerin mixtures are popular: **Lactophenol, Glychrogel, Brandt's** and **Kaiser's** glycerin jellies. Having taken the easiest one to prepare, for their merits are about equal, the tissue is mounted and covered and it is necessary to seal the edges. In the case of temporary mounts a little paraffin applied with a heated scalpel, or wire, will suffice. Lee (p. 230) advocates Peter Gray's sealing medium made up by melting together 4 parts anhydrous lanolin, 1 part Canada balsam (dry) and 8 parts resin which becomes solid on cooling. Apply to edges in the same manner as the paraffin. **Kronig's** cement is employed in Bensley's laboratory. **Duco** cement is very worthwhile because it is insoluble in xylol, alcohol and other chemicals used to clean microscopic preparations. Mallory (p. 99) dilutes it with an equal volume of acetone. See **Karo.**

For sections and tissues which are first dehydrated and cleared the investigator must choose the mounting medium best adapted to his purpose from a considerable number proposed of which the following are given elsewhere in this book: **Balsam, Cedar Oil, Clarite, Colophonium, Damar, Diaphane, Euperal, Nevillite, Sandarac, Terpineol Balsam.**

The chief desiderata are a medium which will harden fairly quickly, which will not become acid and bring about the fading of anilin dyes and which will not crack or develop granules. Clarite is competing for first place with balsam; because, to make the balsam neutral and keep it so, is a troublesome job. Directions for its preparation are given by the Bensleys (p. 39). But the balsam ordinarily purchased is satisfactory for hematoxylin and eosin and iron hematoxylin preparations except when the latter are counterstained with an anilin dye. The writer used to employ cedar oil (for immersion objectives), in mounting sections stained by Giemsa's method, which is superior to balsam, but it drys slowly and is not better than clarite. Damar has been recommended for stains likely to fade and colophonium for thick sections of the nervous system for which covers are not used; both however appear to be less valuable than clarite.

Museum specimens require an aqueous mounting medium which preserves colors. See **Color Preservation.** See **Plastics** for museum work.

**Mucicarmine** for mucus, Mayer's (Mallory and Parker in McClung, p. 417). To make up stain, mix carmine, 1 gm.; aluminum chloride, 0.5 gm.; and aq. dest., 2 cc. Heat over flame for 2 min. Color of solution darkens. Add gradually 100 cc. 50% alc. stirring constantly until dissolved. After 24 hrs. filter. Filtrate keeps well. Stain paraffin sections of absolute alcohol fixed tissue in carmine sol. 5–10 min. Wash in water, dehydrate, clear and mount. Mucus is red. When nuclei also are colored red, add few drops 1% aq. sodium bicarbonate to the stain. It is customary to stain cells and nuclei before hand with alum hematoxylin. Bensley (Cowdry's Special Cytology, 1932, p. 203) uses alcoholic chrome sublimate (sat. mercuric chloride and potassium bichromate in 95% alc.) and increases the content of carmine 5 times.

**Mucigen,** intracellular antecedent of **Mucin.**

**Mucihematein** for mucus, Mayer's, Mallory and Parker in McClung, p. 416). Make up: (A) hematein, 0.2 gm.; aluminum chloride, 0.1 gm.; glycerin, 40 cc.;

aq. dest., 60 cc. and (B) hematein, 0.2 gm.; aluminum chloride, 0.1 gm.; 70% alcohol, 70 cc.; nitric acid, 1–2 drops. *A* is advised except when the mucus swells much in which case use *B*. Stain paraffin sections of absolute alcohol fixed tissue 5–10 min. Wash in water. Dehydrate in 95% alc. and in abs. Clear in xylol and mount in balsam. Mucus stains blue. The other materials are colorless. Preliminary coloration with carmine is suggested. Bensley (Cowdry's Special Cytology, 1932, p. 203) used alcoholic chrome sublimate fixation (sat. mercuric chloride and potassium bichromate in 95% alc.) and increased the content of hematein five times.

**Mucin,** one of several glycoproteins found in mucus. See **Mucus** and **Mucicarmine, Mucihematein** and **Mucisudan** stains.

**Mucisudan** is a dye of undetermined composition made by hydrolysis of sudan black B with acetic acid and recommended as a new stain for mucin (Leach, E. H., J. Path. and Bact., 1938, 47, 637–639).

**Mucoproteins.** A method for histological distinction between the chondroitin sulphuric acid protein of connective tissue mucus and the mucotin sulphuric acid protein of epithelial tissues has been worked out by L. H. Hempelmann, Jr., Anat. Rec., 1940, 78, 197–206. Briefly stated toluidin blue in 1:280,000 will stain the former vividly and the latter not at all.

**Mucus** means slime. It is a viscid, stringy material which ordinarily stains with basic dyes and is found in many parts of the body. The chemical composition of mucus is not uniform. It may consist of one of several glycoproteins, called *mucins*, which are by contrast definite chemical substances. The term *mucous* is an adjective describing a cell or tissue which produces or contains mucus. *Mucigen* is the intracellular antecedent of a mucin. Since there are several mucins there are several corresponding mucigens.

Pathologists sometimes divide mucins into two categories, epithelial and connective. The connective tissue type is found in the ground substance of bone, synovial fluid and in other locations. It increases in amount in the myxedema (G. *myxa*, mucus + *oidema*, swelling) of certain thyroid deficiencies as well as in arteriosclerosis and various tumors. The ubiquitous fibroblast is said to be a great former of mucins. Epithelial mucins are produced by epithelial secretory cells. The goblet cells are easily recognized by the fact that the material to be discharged is held in a goblet like expansion of the cell. Other mucous cells can be distinguished from serous or zymogenic cells by several criteria:

1. The nuclei instead of being roughly spherical are often, but not always, pressed against the cell membrane remote from the lumen.

2. The mitochondria are usually of smaller diameter and shorter than in zymogenic cells.

3. The secretion antecedents (Mucigens) of mucous cells are more difficult to see in the fresh state, more labile, and in fixed tissues are metachromatic and can be stained almost specifically with mucicarmine and mucihematein.

See **Mucicarmine** and **Mucihematein** of Mayer.

A simple method for mucus has been described by Lillie (R. D., J. Tech. Methods, 1929, 12, 120–121). Sections of tissue fixed in formalin or in Zenker-formol (Helly) are passed to water. In the case of the latter remove mercury with iodine and sodium thiosulphate as usual. Stain 1 min. in 0.2% aq. toluidin blue. Wash in water. Dehydrate in *pure acetone*, clear in xylol and mount in balsam. Mucus, reddish violet; nuclei, blue; red cells, yellow or greenish yellow. In the case of old formalin material rinse in 95% alcohol before the acetone.

**Müller's Fluid.** Potassium bichromate, 2–2.5 gm.; sodium sulphate, 1 gm.; aq. dest., 1 gm. This was formerly much used for long fixation and mordanting of nervous tissue. See **Chromaffin Reaction, Decalcification, O'Leary's Brazilian Method, Weigert Method.** It is now largely replaced by **Orth's Fluid** which is really formalin-Müller.

**Mumps.** Refractile, eosinophilic bodies in red blood cells are very small first 5–6 days. Increase in size and elongate 7–14 days. (Parsons, H. H., Military Surgeon, 1938, 83, 541–543).

**Muscle,** to distinguish in sections from connective tissue, Dahlgren (McClung, p. 306) suggests Retterer's and Van Gieson's stains, picronigrosine and Unna's orcein to which may be added Mallory's stain. Demonstration of chloride in muscle fibers (Heilbrunn, L. V. and Hamilton, P. G., Physiol. Zool., 1942, 15, 363–374). For contraction bands and wave mechanics, see Carey, E. J., Arch. Path., 1940, 30, 881–892, 1041–1072. A technique for separating nuclei from cytoplasm for analysis is given under **Nuclei.** If microdissection is contemplated the pioneer paper by Kite, G. L., Am. J. Physiol., 1913, 32, 146–164 should be consulted. The experimental production of myocardial segmentation is

described by Saphir, O. and Karsner, H. T., J. Med. Res., 1923–24, **44**, 539–556. Methods of **Maceration** are often useful in the isolation of single fibers. Mitoses can only be induced in exceptional cases (Allen, E., Smith, G. M. and Gardner, W. U., Am. J. Anat., 1937, **61**, 321). An electron microscopic technique for localization of magnesium and calcium is described by Scott, G. H. and Packer, D. M., Anat. Rec., 1939, **74**, 31–45. See **Myosin** and **Purkinje** cells and fibers.

**Museum Specimens,** see **Color Preservation.**

**Myelin,** see various methods for demonstration of **Nerve Fibers.**

**Myeloblasts.** The recognition of these cells is a fine art; because, by definition, they are so little differentiated that the granules characteristic of the 3 types of leucocytes are absent. For contrasting views, dependent largely on whether supravital staining or fixed and stained preparations are used, see Cowdry's Histology, p. 100, also **Leucocytes,** developmental series.

**Myelocytes,** see **Leucocytes,** developmental series.

**Myeloidin** is the term applied to the material of certain spheroidal or cuboidal bodies of wax-like luster present in the bases of retinal pigment cells of monkeys and some other animals but reported as absent in man and said to resemble myelin. For literature see Arey, L. B. in Cowdry's Special Cytology, 1932, **3**, 1218.

**Myocardium.** Method for separation of fiber bundles (Mall, F. P., Am. J. Anat. **11**, 211–266).

**Myofibrils.** The best method is to fix in Zenker's fluid or strong Flemming's mixture and to stain with iron hematoxylin (see Dahlgren in McClung p. 425). Microincineration is useful for the demonstration of minerals.

**Myoglia** is a fine network of fibers associated with muscle cells well demonstrated by **Mallory's Connective Tissue Stain.**

**Myosin** is a protein, present in muscle, the molecules of which are needle-shaped. Cross striations of muscle are thought to depend on their arrangement. In the isotropic (non-birefringent) bands the myosin molecules are believed to be disposed at random and in the anisotropic (birefringent) bands parallel to the length of the fiber (*see* Bourne, p. 30).

**Myriapoda,** see **Parasites.**

**Nadi Reagent** is dimethyl-paraphenylenediamin + $\alpha$ naphthol. Indophenol oxidase catalyses oxidation of nadi to indophenol blue and that of paraphenylene diamin to diamin.

**Nails.** These very interesting structures are seldom examined microscopically despite the fact that changes in them may provide significant clues to the condition of other tissues. They are chiefly made up of stratum lucidum thickened by much eleidin. It is a simple matter to macerate cut finger or toe nails in 40% aq. potassium hydroxide or in concentrated sulphuric acid for a few days and then to isolate the individual cells some of which are nucleated. MacLeod, J. M. H., Practical Handbook of the Pathology of the Skin. London: H. K. Lewis, 1903, 408 pp. gives Heller's method which involves fixation of ungual phalanx for a few days in Muller's fluid, prolonged washing, decalcification for 4–6 days in 1 part nitric acid and 3 parts of water followed by thorough imbedding in celloidin. The sections can then be stained with hematoxylin, gentian violet, safranin or any other of a number of dyes.

**Naphthalene Pink,** see **Magdala Red.**

**Naphthalene Red,** see **Magdala Red.**

**Naphthamine Blue 3BX,** see **Trypan Blue.**

**Naphthamine Brilliant Blue 2R,** see **Dianil Blue 2r.**

**Naphthol Blue R** (CI, 909)—fast blue 3R, Indian blue 2RD, Meldola's blue, new blue R, phenylene blue—An oxazin dye used by Harvey, B. C. H., and Bensley, R. R., Biol. Bull., 1912, **23**, 225–249 as a supravital stain for gastric mucosa. The Bensleys' report that this dye has proved useful in the localization of unsuspected parathyroid and thyroid tissue in experimental animals. After vascular perfusion in a concentration of 1:40,000 of 0.85% aq. sodium chloride the thyroid, parathyroid and lymph nodes become colored intensely blue; whereas other tissues, muscles, salivary glands etc., are colored pale greenish blue.

**Naphthol Green,** see **Naphthol Green B.**

**Naphthol Green B** (CI, 5)—acid green O, green PL, naphthol green—An acid nitroso dye for which a probable formula is given by Conn (p. 42) and which he thinks was the naphthol green used by v. Volkmann, R. and Strauss, F., Zeit. f. Wis. Mikr., 1934, **51**, 244–249, and by Mollier, G., Zeit. f. Wis. Mikr., 1938, **55**, 472–473.

**Y** (CI, 2)—fast printing green, Gambine—An acid nitroso dye apparently not used in histology.

**Naphthol Orange,** see **Orange I.**

**Naphthol Red S, C** or **O,** see **Amaranth.**

**Naphthol Yellow,** see **Martius Yellow.**

**Naphthylamine Pink,** see **Magdala Red.**

**Narcein** (CI, 152). An acid mono-azo dye. Was used by Ehrlich in combination with pyronin and methylene blue or

methyl green to produce a neutral dye (Conn, p. 54). No longer available.

**Nasal Passages.** The *fluid*, when present in unusual amounts can obviously be studied in **Smears.** Nasal clearance depends upon the movement by the cilia toward the pharynx of a mucous sheet (to which foreign materials become attached) over a layer of fluid in which the cilia act as can be demonstrated by the techniques of Lucas, A. M. and Douglas, L. C., Arch. Otolaryng., 1934, **20,** 518–541 and others. Methods for **Mucus** and **Cilia** are given under their respective headings. The wall of the nasal passages exhibits marked regional diversity (Hilding, A., Arch. Otolaryng., 1932, **16,** 9–18). The nasal mucous membrane covering the septum can be removed *in toto* by the dilute acetic acid method (see **Epidermis**) and examined as a whole mount which gives valuable data impossible to secure from the study of sections. Those interested in wound healing would do well to consult a paper by Boling, L. R., Arch. Otolaryng., 1935, **22,** 689–724. An easy and graphic method for visualization of lymphatic drainage is described under **Lymphatic Vessels.** For numerous suggestions as to technique see Proetz, A. Applied Physiology of the Nose. St. Louis: Annals Publishing Co., 1941, 395 pp.

**Nasal Sinuses.** The mechanism of clearance is similar. To make sections of the nasal sinuses, especially the smaller ones, fixation in **Formalin Zenker** is suggested followed by **Decalcification** and **Celloidin Imbedding.** The sections can be stained by the method best adapted to the purpose in mind.

**Nasmyth's Membrane,** see **Enamel** cuticle.

**n-Butyl Alcohol** (prophylcarbinol). Recommended by Stiles (K. A., Stain Techn., 1934, **9,** 97–100) to replace higher concentrations of alcohol in histological technique especially for lightly chitinized insects but also as a routine for vertebrates. After fixation in **Gilson's Fluid** pass the tissues through 35% (ethyl) alcohol ½–1 hr.; 90 cc. 45% alc. + 10 cc. butyl, 2 hrs.; 80 cc. 62% alc. + 20 cc. butyl, 2 hrs.; 65 cc. 77% alc. + 35 cc. butyl, 4 hrs.; 45 cc. 90% alc. + 55 cc. butyl, 6 hrs. to days; 25 cc. abs. alc. + 75 cc. butyl, 6 hrs. to over night; butyl 2 changes several hrs. (or store in butyl if desired). To imbed transfer to mixture of butyl and paraffin and to paraffin. *n* Butyl alcohol is helpful in making permanent preparations of tissues freshly stained with **Methylene Blue,** which see. It should not be confused with **Tertiary Butyl Alcohol.**

**Necrobiosis** was for Minot (C. S., The Problem of Age, Growth and Death. New York, G. P. Putnam's Sons, 1908, 280 pp.) a condition in which the cells continue to live but change their chemical organization so that their substance passes from a living to a dead state. "Here (he says) life and death play together and go hand in hand." The term is current but is of little use because it has no advantage over the word **Necrosis** for the disorganization of death seldom if ever takes place simultaneously throughout the substance of any living thing. See **Dead Cells.**

**Necrosis** (G. *nekrōsis*, a killing). The term is usually applied to indicate the local death of a cell or of group of cells, not that of the body as a whole. Death is defined by Webster and others as the "cessation of life" which merely poses the question of what life is. Perhaps the most fundamental vital phenomenon is the oxygen consumption involved in respiration. This may persist in erythrocytes even after the loss of their nuclei (Harrop, G. A., Arch. Int. Med., 1919, **23,** 745–752). But cells frozen by special techniques do not respire while frozen. They endure in a state of suspended animation (called vitrification) indefinitely. They are not dead since they retain the structural organization, which, when unlocked by increase in temperature, confers renewed vitality (see Luyet, B., C. rend. Soc. de biol., 1938, **127,** 788–789 and many others). Death can therefore be better defined as the disorganization of living matter which makes permanently impossible all vital phenomena. Since the organization of different sorts of living cells is fundamentally different the loss of organization in them is likely also to be different. See various forms of **Degeneration.** In general necrosis of tissue is often evidenced by a breaking up of the nucleus known as caryorrhexis (G. *Karyon*, nucleus, + *rhēxis*, rupture) or by its solution, caryolysis (G. *lysis*, solution). Consequently any good nuclear strain such as hematoxylin or methylene blue is satisfactory. See techniques for **Dead Cells, Necrobiosis.**

**Negative Stains,** for demonstration of bacteria. The organisms show white in a black or colored background (Cumley, R. W., 1935, **10,** 53–56). See **Azo Blue.**

**Negri Bodies.** 1. *Rapid section method* (Schleifstein, J., Am. J. Pub. Health, 1937, **27,** 1283–1285). Fix in Zenker's fluid, wash, dehydrate in dioxan, embed in paraffin, cut at 4 microns, mount, deparaffinize. Flood slides with 1 drop 1:40,000 aq. KOH in 2 cc. stock solution of stain (Rosanilin of Grubler 1.8 gm.,

methylene blue, Nat. Col., 1 gm., glycerol 100 cc. and methyl alcohol 100 cc.). Steam gently 5 min. Rinse in tap water. Decolorize by gently moving in 90% ethyl alcohol until color is faintly violet. Pass quickly through 95% alcohol, absolute, xylol and mount in balsam. Negri bodies deep magenta with dark blue inclusions.

2. *Rapid smear method* (Dawson, J. R., J. Lab. & Clin. Med., 1934–35, **20,** 659–663). Remove brain to be examined as quickly as possible. Cut several small segments (3–4 mm. thick) from Ammon's horn perpendicular to its long axis and place in Petri dish. Cut away adjacent tissue leaving only the horn. Place a segment, cut surface down, on small end of a new 1 in. cork. With wooden applicator, or match, gently wipe peripheral tissue outward and downward. The segment is thus more firmly attached to the cork and the gray matter containing the pyramidal cells bulges upward. Press this gently against a slide (clean and entirely free from grease) held at one end between thumb and forefinger. Repeat 3 or 4 times, starting at end away from fingers, quickly so tissue does not dry. Immediately immerse in abs. methyl alcohol 5 min. or more. Rinse in running water and stain in 2% aq. phloxine 2–5 min. Wash off excess stain in running water and color in Loeffler's alkaline methylene blue, 10–20 sec. Decolorize in 80% ethyl alc., dehydrate in 95% and 2 changes of absolute, clear in xylol and mount in balsam. Handle slides with forceps and avoid danger from contact with tissue throughout process. Pyramidal cells blue, Negri bodies bright red to reddish brown. Time including examination 25 min.

Stovall, W. D. and Black, C. E., Am. J. Clin. Path., Tech. Suppl., 1940, **4,** 8 recommend control of pH in staining with eosin methylene blue (see **Buffers**). Stain with 1% eosin in 95% alcohol at pH 6.0 or more alkaline. Negri bodies pale red. The red is much more intense if the pH is 3.0. **Loeffler's** methylene blue is best as counterstain at pH 5.3. At pH 6.0 it removes eosin.

Azur B is advised for staining of Negri bodies by Jordan, J. H., and Heather, H. H., Stain Techn., 1929, **4,** 121–126; see also **Carbol-Anilin Fuchsin** methylene blue.

**Neisserian Infection.** A differential stain favorable for diagnosis (Scudder, S. A., Stain Techn., 1931, 6, 99–105).

**Neisser's Stain** for **Diphtheria Bacilli,** which see.

**Nemathelminthes** is the phylum of round worms. See **Parasites.**

**Nematodes.** See **Glychrogel** for mounting. See **Parasites.**

**Neoprene,** injection of blood vessels (Lieb, E., J. Tech. Methods, 1940, **20,** 50–51). Neoprene is a colloidal, finely divided suspension of synthetic chloroprene in an alkaline aqueous medium. Instructions for the human kidney. Cannulate renal artery and wash with tap water at slow but constant rate. Ligate grossly leaking vessels. Continue 8–18 hrs. until organ is pale gray. Cover and keep in ice box 6–7 hrs. or until the next day. Keep specimen at room temperature about one hour before injection. If it feels cold warm it with tap water. Connect cannula with bottle containing neoprene. A special apparatus for maintenance of 150–160 mm. Hg. is advised by Lieb but it is probably sufficient to provide gravity pressure by raising the bottle 5 ft. or more. Close vessels ejecting the neoprene with hemostats and tie them when vessels are completely filled. Rinse in warm water. If a corrosion specimen is wanted leave kidney in conc. commercial HCl in tightly covered vessel at 56°C. over night. Next morning pour off acid and allow stream of water to flow over the cast itself in the bottom of the container. When all debris is removed examine under water with dissecting microscope. Store in 0.3% Dowicide sol. (American Anode Inc., 60 Cherry St., Akron) to avoid mold. Lieb gives more details and describes combined corrosion, histological and roentgenological methods. Technique should be adapted to other organs.

**Nerve Endings.** These may be demonstrated in many ways. Nothing will adequately take the place of their study *in vivo* (Speidel, C. C., J. Comp. Neur., 1942, 76, 57–73); but no method should be used with expectation of satisfactory results the first time. Experimentation is required. Most of the silver methods for neurofibrils show nerve endings. The writer has obtained good results by **Bodian's Method** applied to paraffin sections of experimental tumors. **Graven's Gold Chloride** method may be tried. For silver impregnation of intracellular nerve endings in pars intermedia of pituitary, see Tello, F., Trab. d. Lab. Rech. Biol. Univ. Madrid, 1912, **10,** 145–183. Methylene blue is, since the time of Ehrlich, a very popular stain for nerve endings. Addison (McClung, pp. 477–480) has given a full account of the technique. Commission Certified zinc-free methylene blue is suggested. Dye can be applied locally or by vascular perfusion.

1. *Local application.* Place tissue in

shallow dish on thin layer of glass-wool moistened with 0.1–0.05% methylene blue in physiological salt solution. Add enough stain every few minutes to keep tissue moist and covered by film of stain. Beginning after 15 min. examine frequently at low magnification until nerves are colored blue. Fix stain by immersion in cold 8% ammonium molybdate in physiological salt solution or Ringer's (½ hr.). Wash in cold water. Dehydrate in alcohols in refrigerator a little above 32°C. Either clear in xylol and mount in balsam or imbed in paraffin and section. Cole (E. C., J. Comp. Neurol., 1925, **38**, 375–387) proceeded much in this way. He immersed whole alimentary tract of frog in 1:10,000 methylene blue solution for 1 hr. and cut it in pieces.

2. *Vascular perfusion.* Insert cannula in main artery leading to the tissue. Inject 1:10,000 methylene blue in physiological saline until tissue becomes light blue. Leave 15 min. Remove thin pieces or slices. Place in dish and moisten with methylene blue solution. Examine uncovered at low magnification at intervals until nerve fibers and endings are stained. It is essential as in local application not to exclude air from tissue by covering with too much fluid. Fix in ammonium molybdate and continue as described above. For large fetuses use Langworthy's method (O. R., J. Comp. Neurol., 1924, **36**, 273–297), for the lungs of rabbits that of Larsell (O., J. Comp. Neurol., 1921, **33**, 105–131), for arteriovenous anastomoses Brown's (M. E., Anat. Rec., 1937, **69**, 287–295), and for skin Weddell's (G., J. Anat., 1940–41, **75**, 441–446). Staining may perhaps be accentuated by hydrogen acceptors, see **Auerbach's Plexus.** See **Pacinian Corpuscles, Meissner's Corpuscles, Krause's End Bulbs, Motor End Plates, Boutons Terminaux** and **Synapses.**

**Nerve Fiber Degeneration.** The standard techniques are the **Marchi Method** by which the lipids produced by degeneration are blackened with osmic acid and the staining of lipoids by **Sudan III.** In addition 3 other much quicker methods are recommended:

1. To stain vitally with *neutral red* (Covell, W. P. and O'Leary, J. L., J. Tech. Meth., 1934, **13**, 92–93). Intensity of staining of degenerating myelin depends upon amount and concentration of the dye. It can be applied in 3 ways: (1) Inject 4 cc. 4% neutral red in physiological salt solution into marginal ear vein of a rabbit over period of 1 hr.; (2) Perfuse through aorta with large volume of 1:1,000 solution; (3) Immerse finely teased piece of degenerated nerve in 1:10,000 solution for about 12 min. Vital staining permits immediate determination of extent and degree of degeneration. See the author's excellent colored figures.

2. To examine by *polarized light* (Weaver, H. M., J. Lab. & Clin. Med., 1940–41, **26**, 1295–1304). Lay excised nerves without stretching on piece of wooden tongue depressor and fix 24 hrs. or more in 10% neutral formalin. Cut longitudinal frozen sections 10 microns thick. Float them onto slides from water, mount in neutral glycerin and examine. Weaver gives diagrams to aid in interpretation of findings. See also Pritchett, C. O. and Stevens, C., Am. J. Path., 1939, **15**, 241–250; Radhakrishana, Rao, M. V., Ind. J. Med. Res., 1938, **26**, 103–106.

3. To demonstrate early changes in the axis cylinders (cores of the fibers) *Alzheimer's modification* of Mann's eosin-methyl blue method is strongly recommended by Mallory as showing normal axis cylinders deep blue and degenerated ones, red.

**Nerve Fibers.** Many excellent methods present themselves: the continuous direct observation of the growth of individual fibers in living tissues of lower animals (Speidel, C. S., Biol. Bull., 1935, **68**, 140–161); the microdissection of living fibers (De Renyi, G. S., Cowdry's Special Cytology, 1932, **3**, 1370–1402); x-ray diffraction studies of the sheaths (Schmitt, F. O., Bear, R. S. and Palmer, K. J., J. Cell. and Comp. Physiol., 1941, **18**, 31–42) and microincineration (Scott, G. H., Proc. Soc. Exp. Biol. & Med., 1940, **44**, 397–398). For their demonstration in fixed tissues consult methods of **Bodian, Davenport, Golgi, O'Leary, Osmic Acid, Weigert** and **Weil.** The methylene blue technique of staining nerve fibers is given under **Auerbach's Plexus.** See **Nerve Endings, Motor End Plates, Bouton Terminaux.**

**Nerve Grafts,** methods, histological and otherwise (Sanders, F. K., and Young, J. Z., J. Anat., 1942, **76**, 143–166).

**Nerve Plexuses,** see **Auerbach's.**

**Nervous System.** This, the most complicated of bodily parts, can be investigated microscopically in a great many different ways. It is however shielded from the environment so that there are great obstacles in the way of direct observation *in vivo*. In mammals the best that can be done is to insert windows in the wall of the skull. A technique for this purpose, designed by Forbes (H. S., Arch. Neurol. and

Psychiat., 1928, **19**, 75), permits direct study at low magnification of blood vessels with so little injury that their behavior in various experimental conditions can be investigated. It is likely that by the **Sandison Technique** very significant observations can be made on living, growing nerve fibers of the rabbit. In amphibia Speidel (C. S., Biol. Bull., 1935, **68**, 140–161) has been particularly successful in devising methods for study of nerve fibers *in vivo*.

Another group of techniques is available for *marking in vivo* and examination of the tissues after removal. **Vital Staining** has been much used. Some factors that condition the coloration of nerve cells with trypan blue have been described by King, L. S., J. Anat., 1934–35, **69**, 177–180. The pathways of drainage of cerebrospinal fluid can be marked with **Prussian Blue** (Weed, L. H., J. Med. Res., 1914, **26**, 21–117). Nerve fibers and cells can of course be marked by the *in vivo* creation of injuries and subsequently examined. To determine the distribution of **Radiophosphorus** may prove helpful.

For the examination of excised tissues a host of methods present themselves. Consider first the classical techniques from which several others spring.

1. The original Nissl method for *internal structure* of the nerve cell consisted of fixing in alcohol and of staining sections with methylene blue. It revealed a basophilic material called **Nissl Substance.** The unfortunate tendency now-a-days is to loosely designate all methods intended to demonstrate this substance as Nissl techniques even though resemblance to the original method is lacking.

2. The original Golgi method for the *external form* of nerve cells depends upon preliminary mordanting of tissue in potassium bichromate solutions, followed by immersion in weak aqueous silver nitrate, and the cutting of thick sections in which occasional nerve cells and processes are outlined with startling clarity by the black deposit of silver chromate. Cajal modified and speeded up the technique by addition of osmic acid to the bichromate solution (see **Golgi Method,** quick). But the most used modification is the **Golgi Cox** technique.

3. The original **Weigert** method for *myelin sheaths* of nerve fibers depended likewise upon preliminary mordanting in bichromate and the formation of hematoxylin "lakes" when the sections were later stained with hematoxylin. Its most important modification is known as **Weigert-Pal.** The **Marchi** method, as modified by Swank and Davenport is based on similar mordanting with bichromate after which they are treated with osmic acid and was designed to reveal degenerated myelin sheaths the lipids of which are unaffected by the mordanting and are blackened while those of the normal sheaths are not.

4. Cajal and Bielchowsky introduced valuable methods for *axones*, *neurofibrils*, and *nerve endings* including synapses. Both techniques as applied to blocks of tissue depend on preliminary "silvering" with weak silver nitrate solution but in those of the former the silver is reduced by a photographic developer generally hydroquinone or pyrogallic acid; while in those of the latter the tissues are first brought into an ammoniacal silver solution and then reduced in formalin. The most useful modification is the **Bodian Method** of activated protargol. See its evolution under **Silver Methods** which are of assistance in the study of many other tissues of the body as well as the nervous system.

5. Weigert's *neuroglia* stain was also a classic, likewise Cajal's gold chloride and sublimate method (1913) which was soon followed by Hortega's carbonate silver method (1917). See recent techniques under **Neuroglia.**

There are still other techniques to choose from which are not so directly developments of the neurological classics. Nerve cells are closely mixed with fibers. To isolate them sufficiently for direct study at high magnification in approximately isotonic media involves considerable injury and they cannot be held under observation for long periods because their death ensues fairly quickly. Spinal ganglion cells are the easiest studied. The **Maceration** technique is not much used for the nervous system but Addison (McClung, p. 439) states that, if pieces of the anterior horn of the spinal cord are treated with Gage's dissociator (0.2% formalin in physiological saline) for 2–3 days, the nerve cells can easily be dissected out under a binocular microscope, stained and examined more or less as units. **Tissue Culture** of nerve cells of the adult is not feasible because they are fixed postmitotics (having permanently lost the power of multiplication); but culture of young tissues provides interesting results (Levi, G., Arch. de Biol., 1941, **52**, 1–278, profusely illustrated). **Nerve Fibers** are more easily isolated and their investigation in the fresh state is very profitable.

The histological localization of **Cholinesterase** is now feasible. The measurement of oxidative metabolism in different parts of the nerve cell by reduction of ferric chloride (Gerard, R. W., Assoc. for Res. in Nerv. & Ment. Dis., Baltimore, Williams & Wilkins, 1938, **18**, 316–345) can probably be tied up with localization of **Oxidases** and **Peroxidases.** Marinesco (G., Arch. Suisse de Neurol. et de Psych., 1924, **15**, 1–24) has published repeatedly on these enzymes in nerve cells. Methods for **Pigments** and **Lipids** can easily be applied to the nervous system. For microincineration of nerve cells and fibers see Scott, G. H., Proc. Soc. Exp. Biol. & Med., 1940, **44**, 397–398. If it is desired to demonstrate mitochondria the **Anilin-Fuchsin Methyl Green** method is suggested after fixation by vascular perfusion plus immersion. See in addition to above headings: **Auerbach's Plexus, Axis Cylinders, Boutons Terminaux, Centrosomes, Cresyl Violet, Golgi Apparatus, Microglia, Motor End Plates, Nerve Endings, Neurofibrils, Neurosecretory Cells, Oligodendroglia.**

**Neurofibrils.** These delicate fibrils and networks can be demonstrated with difficulty mainly by methods of silver impregnation in the cytoplasm of nerve cells. In the living nerve cells of selected invertebrates they can also be seen but opinion is divided as to whether they can be detected in the living nerve cells of vertebrates (Cowdry, p. 393).

None of the techniques for neurofibrils are really satisfactory, but, with patience, fairly good results can be secured of adult nerve cells by the following modification (Cowdry, E. V. Internat. Monatssch. f. Anat. u Physiol., 1912, **29**, 1–32) of Cajal's technique. Fix pieces not more than 2 mm. thick in Carnoy's 6:3:1 fluid 2–6 hrs. Wash in aq. dest. 24 hrs. 1.5% aq. silver nitrate at 39°C. for 3 days with one change. Rinse in aq. dest. and reduce in pyrogallic acid 1 gm.; aq. dest., 100 cc.; formalin 5 cc. in the dark, 24 hrs. Wash in aq. dest. 1 hr. Dehydrate 1 hr. in 95%; 2–4 hrs. in abs. changed twice; clear in cedar oil, 2 hrs.; imbed in paraffin 2 hrs. Rinse deparaffinised sections in aq. dest. 0.1% aq. gold chloride neutralized with lithium carbonate 2 hrs. The sections take a dark purple black color. 5% aq. sodium hyposulphite 5 min. to bleach out excess of silver. Rinse in aq. dest. dehydrate, clear in toluol and mount in balsam.

The neurofibrils are exaggerated optically by their sharp blue black stain in a colorless background. Moreover they form centers for the deposit of silver which probably increases their bulk. The Nissl bodies can be brought out by staining in the usual way with toluidin blue after washing in aq. dest. following treatment of the sections with sodium hyposulphite. The essential step in this technique is the impregnation with silver. Consequently the time in the silver solution should be varied and perhaps its concentration likewise. To obtain a good preparation without many trials is not to be expected.

Silver techniques for neurofibrils are legion. A book has been written on the subject (Cajal, S. R. and deCastro, F., Elementos de Tecnica micrografica del sistema nerviosa. Madrid, 1933). Special methods are advised for different parts of the nervous system and for animals of different sorts and ages. A very useful synopsis is given by Addison (McClung, pp. 452–466). See also Seki, M., Ztschr. f. Zellf. u. Mikr. Anat., 1939–40, **30**, 548–566.

**Neuroglia.** This is the connective tissue of the nervous system. Like that of the rest of the body it consists of cells, fibers (or fibrils as they are called) and intercellular substance. The last named is inconspicuous and little known. The **Neuroglia Fibrils** are considered separately. The cells are of three principal sorts: (1) microgliocytes of mesenchymatous origin. These may be resting and extend long, delicate processes or they may be ameboid in which case they look something like lymphocytes being usually identifiable by intensely staining nuclei. (2) astrocytes (star cells) and (3) oligodendrocytes (little tree cells) both of ectodermal origin. A tabular comparison of the three is given in Cowdry's Histology, p. 406. No neuroglial cells possess Nissl bodies. See **Cajal's Brom-Formol-Silver Method,** the **Phosphotungstic Acid Hematoxylin** method of Mallory, Weil and Davenport's silver methods given under **Microglia** and **Oligodendroglia** and **Alzheimer's Modification** of Mann's eosin-methyl blue method.

**Neurosecretory Cells.** A good deal has been written on the subject. The most recent data on location in nervous system and methods are provided by Scharrer, E., J. Comp. Neurol., 1941, **74**, 87–92; Scharrer, B., *ibid*, 93–130.

**Neutral Fats.** These are glycerides of fatty acids. See **Lipids,** examination of with polarized light. Colored rose red by **Nile Blue Sulphate.** See **Sudan Stains, Osmic Acid** and **Oil Red O.**

**Neutral Gentian** (Bensley, R. R. Am. J.

Anat., 1911, **12**, 297-388). This gives a very fine deep violet coloration of secretion antecedents of serous (or zymogenic) cells. It has been used particularly for the pancreas and the stomach.

Neutral gentian is the neutral dye obtained when aq. gentian violet (crystal violet) is precipitated by its equivalent of aq. orange G which is added slowly and the mixture agitated. Use solutions almost but not quite saturated. If the right amount of orange G solution is added almost complete precipitation is obtained. If too much is added the precipitate is dissolved in which case add more gentian violet. Excess of orange G can be detected by the production of a yellow ring of stain about a violet center when a drop of the solution with the precipitate is touched to a piece of filter paper. When satisfied that ppt. is maximal, filter; and dissolve dried ppt. in 20% alc. until "color of a good haemalum solution is obtained". Allow the solution to stand 24 hrs. before use.

*Fixatives:* Several are advised. (1) Equal parts sat. alc. mercuric chloride and 2.5% aq. potassium bichromate. (2) Potassium bichromate 2.5 gms.; mercuric chloride, 5 gms.; aq. dest., 100 cc. (3) Zenker's fluid less acetic 90 cc., neutral formalin 10 cc. or (4) 2% osmic acid 2 cc.; 2.5% potassium bichromate 8 cc.; glacial acetic acid 1 drop. In the case of the last the paraffin sections are treated with 1% aq. potassium permanganate 1 min.; 5% aq. oxalic acid 1 min. and are washed thoroughly in water before staining. Stain 4$\mu$ sections 24 hrs. Blot with several layers filter paper. Dehydrate in acetone. Place in toluol. Differentiate in 1 part abs. alc. and 3 parts oil of cloves. Wash in toluol and mount in balsam. Zymogen granules, purple; cytoplasm and nucleus, yellow; chromophile material, lavender.

**Neutral Red** (CI, 825)—toluylene red—This weakly basic amino-azin dye is used for many purposes. It is a chloride. Some advocate the iodide as more easily purified but neutral red sold by any reliable manufacturer is satisfactory. Vital neutral red is recommended by Conn. The principal uses of neutral red are to stain:

1. *Islets of Langerhans of the pancreas* (Bensley, R. R., Am. J. Anat., 1911, **12**, 297-388). Add 2 cc. of a previously prepared 1% aq. neutral red to 300 cc. physiological salt solution (0.85% NaCl) thus making a concentration of neutral red of 1:15,000. Place this, and as much more as may be required in a bottle from the bottom of which a glass tube leads off, or in an ordinary bottle with a bent glass tube to serve as a siphon. The tube is connected with a glass cannula by about 5 feet of rubber tubing. A freshly killed guinea pig is bled from the throat. Insert the cannula in the thoracic aorta and inject the solution by raising the bottle to a height of 4 or 5 feet. Expose the pancreas. Cut the inferior vena cava near the heart so that the blood, followed by the solution, can easily escape. The pancreas will take on a deep rose red color. Remove pieces, mount in physiological salt solution under cover glasses and examine at low magnification. The optimum depth of staining must be determined experimentally. The islets of Langerhans appear as deep yellow red irregular masses of different sizes in a pale red background. After a time the dye is bleached from the background and the islets become more sharply stained.

A wonderfully fine color contrast can be secured when methylene blue is added to the neutral red solution in a concentration of 1:10,000 and both are injected in the same way. The islets are stained yellow red and the ducts blue. But it is desirable first to obtain satisfactory results with the methylene blue alone.

2. *Parietal cells in the stomach,* (Harvey, B. C. H. and Bensley, R. R., Biol. Bull., 1912, **23**, 225-249). These are beautifully stained by injection with neutral red as described above.

3. *Granules in blood cells.* Touch a drop of fresh blood to a little 1:15,000 neutral red on a slide and cover immediately without attempting to mix. When the size of the drop of blood and the amount of stain are properly estimated the cover glass will press out the fluid into a thin film suitable for examination. The specific granules of leucocytes are stained red. In the monocytes red stained granules appear and sometimes increase in size. When the staining is fairly intense, or after a sufficient interval the nuclei of the leucocytes become colored and also a basophilic material in young reticulated red blood cells. Simultaneous coloration with **Neutral Red** and **Janus Green** is frequently carried out by hematologists.

Fluorescent X is a special type of reduced neutral red (Lewis, M. R., 1935, **17**, 96-105). See **Nerve Fiber Degeneration** and **Nissl Bodies.**

**Neutral Red and Janus Green.** These are often employed together as a supravital stain for blood cells. A recent comprehensive statement of the technique

is given by Cunningham and Tompkins (Downey, pp. 555–579). They add 3 drops conc. janus green in absolute alcohol to 1 cc. dilute neutral red, which, latter, is 20–30 drops conc. neutral red in absolute alcohol. This mixture is spread evenly on slides and evaporated. They caution that for exudates, tissue scrapings, leucemic blood, bone marrow and lymph nodes it is necessary to use stronger solutions. Neutral red C.C. (Commission Certified) is satisfactory in place of the neutral red-iodide advised by Sabin. Fresh blood is mounted on the dye deposit, and is ringed with vaseline to prevent evaporation. This technique has had a profound influence on cytology. Obviously it must be cautiously used and observations discontinued as soon as evidences are seen of experimental modifications in the cells. It affords valuable information on the mitochondria and neutral red granules not stainable together by other methods, but it will not supplant the staining of blood smears by the methods of Giemsa, Wright and others. See critical evaluation by Hall (Downey, pp. 643–698). See application in study of lymphosarcomata (Hu, C. H. and Pai, H. C., Arch. Path., 1942, **34**, 106–116).

**Neutral Red Iodide.** This is a special form of neutral red prepared by Phillips, M. and Cohen, B., Stain Techn., 1927, **2**, 17–18 and recommended by Sabin for the **Neutral Red Janus Green** method.

**Neutral Safranin,** or **Safranin-acid violet** (Bensley, R. R., Am. J. Anat., 1911, **12**, 297–388). Make the neutral dye by precipitating sat. aq. safranin O with sat. aq. acid violet. The latter is added slowly and the mixture is agitated gently. The precipitation should be complete so that when it settles the supernatant fluid is of a faintly violet color. Filter and dissolve dried ppt. in abs. alc. Dilute this stock solution with equal vol. aq. dest. allow to stain 30 min. before use. Stain sections, fixed as described under **Neutral Gentian,** in the same way as with neutral gentian. Nuclei are colored with safranin and secretion antecedents with the acid violet. The method has been used chiefly for the pancreas but it gives fine coloration of nerve as well as gland cells. Unfortunately the colors are not very permanent.

**Neutral Stains.** As explained by the Bensleys (p. 65) acid and basic dyes are mutually antagonistic. One will extract the other from a section. This can be overcome by having them react on each other to form a molecularly balanced neutral compound insoluble in pure water and which must therefore be employed in alcoholic solution. Because the staining depends upon the hydrolytic splitting of the compound they must be applied at maximum concentration of water consistent with retaining the dye in solution. It is on account of the necessity for dilution with water to promote dissociation that water is added to Wright's blood stain on the slide. These neutral dyes are of particular value in the staining of secretion antecedents by R. R. Bensley and his followers, see **Neutral Gentian** (gentian violet-orange G), **Neutral Safranin** (safranin-acid violet), **Crystal Violet-Acid Fuchsin** and **Bowie's Stain.**

**Neutrophile Leucocyte** (finely granular leucocyte, polymorphonuclear leucocyte). Most numerous granular leucocyte, percentage 55–75; slightly smaller (9–12$\mu$) than eosinophile; nucleus lobated, usually also filamented, stains deeply; specific granules, refractile, neutrophilic, small, uniform and numerous; highly motile and phagocytic. Special methods for their study are far too numerous even to list. The so-called toxic neutrophiles in certain pathological states differ from normal ones in the staining of nuclei and specific granules (Mommsen, H., Ztschr. exper. Med., 1929, **65**, 299). A comprehensive account of neutrophiles is provided by Bunting, C. H. in Downey's Hematology, 1938, **1**, 160–177. Because these cells normally constitute by far the majority of leucocytes in the circulating blood, chemical analyses of total leucocytes separated from the erythrocytes relate chiefly to them. The most convenient way is to mix fresh blood with **Anticoagulant,** centrifuge and take the so-called buffy layer. For lipid analysis of such material, see Boyd, E. M., Arch. Path., 1936, **21**, 739–748. Another useful method, described by Haan and employed by Barnes, J. M., Brit. J. Exp. Path., 1940, **21**, 264–275, which works nicely with the rabbit but poorly with the cat, is to inject intraperitoneally 200–300 cc. warm sterile saline solution and 4 hrs. later to withdraw fluid with a cannula into 5 cc. 4% sodium citrate. This fluid contains 95–98% neutrophiles. Barnes has outlined methods for determination of **Cathepsin, Nuclease, Amylase, Lipase, Lysozyme** and **Adenoinase.** Since it is possible now to break up cells and to collect by centrifugation masses of **Mitochondria** and **Nuclei,** it should be feasible to collect and similarly to analyse the neutrophilic granulations. For technique of meas-

uring motility, chemotaxis and other properties, see **Leucocytes.**

**Neutrophilic,** see **Staining.**

**Nevillite V** and No. 1 have been compared with gum damar and Canada balsam as mounting media by Groat (R. H., Anat. Rec., 1939, **74**, 1–6). Both are clean, colorless, inert and neutral. He recommends a 60% solution of either V or No. 1 in toluol.

**New Blue R,** see **Naphthol Blue R.**

**New Fuchsin** (Magenta III) (CI, 678)—fuchsin NB, isorubin—It is triaminotritolyl-methane chloride. This new fuchsin is sometimes specified for staining of acid fast bacilli.

**New Methylene Blue.** The Colour Index lists several dyes by this name of which 2 deserve mention: (1) **GG** (CI, 911) is recommended by the Bensleys (p. 16) as a supravital stain for mast cells and for the thyroid because of its metachromatic capacity. (2) **N** (CI, 927)—methylene blue NN—Conn (p. 88) says that it may be of some value though it is practically never used in microscopical work. Cowdry tried it and found that it had no particular advantages.

**New Pink,** see **Phloxine.**

**New Ponceau 4R,** see **Ponceau 2R.**

**New Victoria Blue B** or **R,** see **Victoria Blue R.**

**New Victoria Green Extra O, I** or **II,** see **Malachite Green.**

**Niagara Blue 3B,** see **Trypan Blue.**

**Niagara Blue 4B** (CI, 520)—benzo sky blue, direct sky blue, pontamine sky blue 5BX—A disazo dye, see Varrelman, F. A., Stain Techn., 1938, **13**, 115–119. Niagara blue 2B (N.A.C.) is the American prototype of trypan blue for which it can be substituted (Foot, McClung, p. 115).

**Nickel.** The microchemical technique of Crétin and Pouyanne (A., and L., Bordeaux chirurgical, 1933, **4**, 321–364) employed in a study of the influence of metals on bone deposition, as given by Lison (p. 102), is: Fix in formol, 30 cc., "sérum physiologique", 100 cc., and ammonium hydrosulphate 5 drops. Immerse in a solution of ammonium phosphate in order to produce the insoluble double salt: $NH_4NiPO_4 + 6H_2O$. Decalcify. In the sections stain the nickel by an alcoholic solution of pure hematoxylin which forms a lilac colored nickel lake appearing blue when very thick (Lison, p. 102).

**Nigrosin,** water soluble (CI, 865)—gray R, B, BB, indulin black, silver gray, steel gray—Commission Certified. This is a mixture. It has been used as a counterstain for neutral red in coloration of Nissl bodies by Bean, R. J., Stain Techn., 1927, **2**, 56–59, as a negative stain for bacteria, etc. See **Picro-Nigrosin.**

**Nile Blue A,** see **Nile Blue Sulphate.**

**Nile Blue Sulphate** (C 1. 913)—Nile Blue A—This is an important oxazin dye for which purity tests have been established (Conn, p. 270). It was introduced by Lorrain Smith as a fat stain. Briefly the method is to stain fresh tissues, or frozen sections of formalin fixed tissues, for 10–20 min. in a conc. aq. solution of Nile blue sulphate, to differentiate in 1% aq. acetic acid, wash in water and mount in glycerin. He thought that the *neutral fats* (*glycerides*) were thereby colored red and the *fatty acids* blue, but Kaufmann and Lehmann (C. and E., Virchow's Arch. f. Path. Anat. und Physiol., 1926, 261, 623–648) came to the conclusion that the method was valueless. However Lisson (p. 202) was unimpressed by their evidence. In his opinion the rose (or red) color does signify the presence of a nonsaturated glyceride whereas the blue color is of no significance because of its lack of specificity. He reported that some mixtures of free fatty acids remain uncolored; for those containing saturated fatty acids non-coloration is the rule; while some others, not containing fatty acids, are colored. See **Lipids,** tabular analysis.

**Nile Pink,** fat stain prepared from nile blue sulphate by boiling with dilute sulphuric acid (Rettie, T., J. Path. & Bact., 1931, **34**, 595–596).

**Ninhydrin Reaction.** Berg's (W., Pfluger's Arch., 1926, 214, 243–249) directions: Fix tissues in 10% formalin, wash in water. Boil section for 1 min. in 2 cc. 0.2% ninhydrin. Wash, mount in glycerin or glycerin jelly. Amino acids, polypeptides and proteins blue or violet. Romieu (M., Bull. d'Hist. Appl., 1925, **2**, 185–191) employs a strong solution heated less. See Giroud (A., Protoplasma, 1929, **7**, 72–98).

**Nissl Bodies** (Tigroid bodies, chromophile granules, chromidia, etc.) are masses of basophilic material easily demonstrable in the cytoplasm of most nerve cells after a wide variety of fixations. Certain types of nerve cells are characterized by the shape, number, size and distribution of their Nissl bodies. Since, moreover, the Nissl bodies appear at a definite stage in the development of the cells and undergo distinctive modifications in physiological and pathological conditions there can be no question that they represent material present *in vivo* although they cannot be distinguished as such in living nerve cells. Bensley, R. R. and Gersh, I.,

Anat. Rec., 1933, 47, 217–237 claim that their discovery of well-formed Nissl bodies, stainable with toluidin blue, in sections of tissues frozen in liquid air and dehydrated *in vacuo* while still frozen is proof of the presence of Nissl bodies in the living state. Wiemann, W., Zeit. f. d. ges. Neurol. u. Psychiat., 1925, 98, 347–404 appears to have made ultraviolet photomicrographs of Nissl bodies, and a dense ash, revealed by microincineration (Scott, G. H., Proc. Soc. Exp. Biol. & Med., 1940, 44, 397–398), corresponds with them topographically.

The influence of *fixation* on the shape (and perhaps to a slight degree on the distribution) of Nissl bodies in nerve cells has never been clearly defined. It is known that the Nissl bodies are much more pronounced after fixation in 95% alcohol, Zenker's fluid and Carnoy's fluid than they are after fixation in osmic acid, Altmann's fluid and Regaud's fluid. Fixatives of the first group also result in more stainable particles in the nucleoplasm than those of the second. For other details see Hopkins, A. E., Anat. Rec., 1924, 28, 157–163. Influence of *staining* is also a factor to be reckoned with because of the striking difference in appearance of Nissl bodies when intensely and lightly colored. There are many methods from which to make a choice. Some of these are given under **Gallocyanin, Gallamin Blue** and **Carbol-Fuchsin.** See also the methods of **Huber, Johnson** and **King.** An apparatus has been devised apparently suitable for obtaining the **Absorption Spectra** of Nissl bodies.

**Nitrates.** Make frozen sections of fresh tissues. Cover section on a slide with 1–2 drops hot 10% "Nitron" in 5% aq. acetic acid. Place in refrigerator 30 min. to permit nitrates to crystallize and examine in polarized light. Nitron is diphenyl-endo-anilo-dihydriazole. It precipitates nitrates as insoluble salts (Crämer, G., Zbl. allg. Path., 1940, 74, 241–244).

**Nitrazine**—nitrazine yellow, delta dye indicator—An acid mono-azo dye suggested as substitute for ponceau de xylidine in **Masson's Trichrome Stain.**

**Nitrazine Yellow,** see **Nitrazine.**

**Nitrocellulose** for imbedding. Low viscosity nitrocellulose ("Hercules Powder Co.'s R.S. 0.5 second nitrocellulose") does not require to be washed as in the case of celloidin. First add absolute alcohol, break up lumps and add ether. Use 100 gms. nitrocellulose, 100 cc. absolute alcohol and 140 cc. anhydrous ether. For evaporation a large surface is required in proportion to depth. A precision microtome is needed for sectioning blocks after first hardening in 70–80% alcohol. Blocks are cut both dry and wet. Serial sections 4 microns thick are obtainable whereas in celloidin the minimum is about 12 microns. Since low viscosity nitrocellulose (L.V.N.) is more readily dissolved than celloidin by absolute alcohol the use of butyl alcohol between 95% alcohol and xylol is suggested (Davenport, H. H. and Swank, R. L., Stain Techn., 1934, 9, 137–140).

**Nitro Dyes.** Chromophore-$NO_2$. All strongly acid. Aurantia, martius yellow, picric acid.

**Nitro Reaction** to distinguish between pyrrols and indols. Treat preparation with a mixture of sulphuric and nitric acids (equal parts). Substances containing the benzene ring (and among them indol compounds) are nitrified and recognizable by their canary yellow color whereas the pyrrols are not nitrified (Lison, p. 162). See Lison, L., J. Physiol. et Path. Gén., 1933, 31, 82–99).

**Nitroprusside Reaction** for **Glutathione.** 1. Mattei and Dulzetto (Atti. e. rend. della Accad. dei Lincei, 1928, 8, 317). Fix in 20% trichloracetic acid. Treat frozen sections 3–4 min. with a fresh solution of sodium nitroprussiate. After quickly drying expose to $NH_3$ vapor. Freeze solidly with ice or solid $CO_2$. Examine frozen on slide at 5°C. The violet color of sulphydryl rapidly disappears.

2. Joyet-Lavergne (Ph., Bull. d'Hist., 1928, 5, 331–349) Method 1: apply to tissue 1 drop 5% aq. sodium nitroprussiate, then 1 drop ammonia and examine immediately. Method 2: before applying reagent as above he uses a stimulant 10% aq. potassium cyanide, 5 min.; or 2% aq. sodium sulphite, 10 min., or sat. ammonium sulphate, 15 min., or trichloracetic acid, 2–5 min. Method 3 for fixed tissues: fix several hours in abs. alc. or in formol 15 cc. + physiological saline sol. 75 cc. Tease tissue or make frozen sections. Stimulate with potassium cyanide or ammonium sulphate. Then apply reagent.

3. Giroud and Bulliard (A. and H., Protoplasma, 1933, 19, 381–384). Apply to fresh teased tissues or frozen sections 10% aq. sodium nitroprussiate alkalinized by about 2% ammonia. Fix the color by treatment for several seconds with 5% aq. zinc acetate. Dehydrate, clear and mount in balsam in the usual way. The violet color becomes red but lasts some time especially if kept in ice

box. The same technique is possible after alcohol fixation.

Lison (p. 135) has considered the specificity of these reactions and recommends analysis given in an article by Rapkine contained in the last edition of Langeron's *Précis de Microscopie.* For fresh tissues (pieces, smears, frozen sections) (a) *Glutathione reduced.* Add to tissue on slide 1 drop 5% sodium nitroprusside for plants, 2% for animals. Add a reinforcer such as sat. aq. ammonium sulphate or crystals, then drop of ammonia. Red or violet color. (b) *Glutathione total.* Treat tissue with 10% cyanide of potassium, 5–10 min. Then (a). (c) *SH radicals fixed to proteins.* 10% trichloracetic acid 15 min. Wash in much water. Repeat several times. For fixed tissues avoid employing absolute alcohol or trichloracetic acid. Use instead formol-saline (above). Then follow as for fresh tissues. Fix colors with zinc acetate as described.

Bourne (G., Austral. J. Exp. Biol. & Med. Sci., 1935, **13**, 238–249) puts frozen sections into hot 5% aq. acetic acid 30–90 sec.; drains off the acid; adds 5% sodium nitroprusside (saturated with ammonium sulphate) 2 min., then few drops conc. ammonium hydroxide which turns them purplish blue. For quantitative unreliability of the test for -SH and -S.S- see Hammett and Chapman, (F. S. and S. S., J. Lab. & Clin. Med., 1938–39, **24**, 293–298).

**Nitrosamino Reaction** of Lison (p. 161) consists in transforming the amino group present in pyrrol and indol into nitrosamine by action of nitric acid; then by demonstrating the nitrosamine by the reaction of Liebermann.

**Nitroso Dyes** (quinone oximes). Produced by nitrous acid acting on phenolic compounds. Naphthol green B and Y.

**Nonfilament-Filament Ratio.** This is derived from the **Filament-Nonfilament Count**, the number of nonfilamented neutrophiles being multiplied by 100 and divided by the number of filamented ones. See Stiles, M. H., J. Lab. & Clin. Med., 1940–41, **26**, 1453–1460.

**Nopalin G**, see **Eosin B** or bluish.

**Normal Solutions.** The equivalent of a substance (equivalent weight, the gram equivalent) is the weight in grams which in its reaction corresponds to: a gram atom of hydrogen, or of hydroxyl, or a univalent ion, or to half a gram atom of oxygen. A normal solution contains one equivalent per liter, a 0.05 normal contains 0.05 equivalent.

*Hydrochloric acid* (HCl), the molecular weight is H = 1.008 + Cl = 35.457 (see **Atomic Weights**) = 36.465. Consequently make up 36.465 gms. of HCl to 1 liter with aq. dest. But it can not be weighed out in this way. Since conc. hydrochloric acid (sp. gr. 1.19) is approximately 12 N, to make a normal solution (approximate) dilute 83.3 cc. to 1 liter with aq. dest. The normality can be accurately determined by standardizing with sodium carbonate, or by titration with a solution of sodium hydroxide of known normality.

*Sulphuric acid* is $H_2SO_4$. Molecular weight calculated in the same way is 130.136. But there are 2 replaceable hydrogen atoms so that in making a normal solution the molecular weight is divided by 2 which means that 65.068 gms. of $H_2SO_4$ is to be made up to 1 liter with aq. dest. A conc. sol. (sp. gr. 1.84) is approximately 36 N. To make approximately 1 N dilute 27.8 cc. to 1 liter.

*Oxalic acid* has the formula $(COOH)_2 \cdot 2H_2O$ with molecular weight of 126. Owing to presence of 2 hydroxyl groups it has 2 hydrogen equivalents and it is necessary to divide the molecular weight by 2 so that 63 gms. is made up to 1 liter with aq. dest.

The alkali sodium hydroxide (NaOH) has 1 hydroxyl group, so that the molecular weight is taken without division. But with disodium phosphate, the formula of which is $Na_2HPO_4$, the hydrogen equivalent is $\frac{1}{2}$ $Na_2HPO_4$, so that the molecular weight is divided by 2. Similarly with the salt $Na_2SO_4$ the molecular weight is halved. For sodium triphosphate, $Na_3PO_4$, the hydrogen equivalent is $\frac{1}{3}$ $Na_3PO_4$, or the molecular weight is divided by 3.

**Normality.** Microscopic study of tissues will be of little value in medical research unless their normal structure is at least approximately known as a basis on which to interpret the findings. Unfortunately there is no general agreement as to what constitutes normal and abnormal, but the statistical definition of normality provides at least a working basis. According to it the normal state is the usual one in a homogeneous group. By usual we mean that it is present in the majority, 51% or more, of the individuals. By homogeneous we mean that the individuals are of the same age, sex, race and are living under similar conditions, that, in other words, no factor is to the best of our knowledge operative likely to produce diversity among them in the particular feature the normality of which is under consideration. Thus, if a certain measure of calcification of the wall of the aorta is found in 56% of individuals of a homogeneous group in St. Louis, it must be regarded as normal for them. But it

does not follow that the same grade of aortic calcification is normal for a group of Japanese of the same sex and age in Tokyo. For them an entirely different grade may be normal occasioned by factors of race, environment, etc. not operative in the same way for the St. Louis group. In speaking of normality it is necessary to be very specific. An aorta may be normal in respect to degree of calcification but abnormal, or unusual, in other respects. Consequently the normality of this or any other tissue can only be established for the particular property measured assuming that the technique of observation is adequate and the number of individuals examined is sufficiently large.

**Normals, Gross Sizes.** What these are is only known in a very hazy way. Yet if the size of an organ is distinctly abnormal this fact must clearly be taken into consideration in evaluating the results of its microscopic study. The best way is to search for papers dealing with the organ in which one is interested in the Quart. Cum. Index Med. The older data are summarized by Vierordt, H., Anatomische Physiologische und Physikalische Daten und Tabellen. Jena: Fischer, 1906, 616 pp. A summary of measurements on infants and children is provided by R. E. Scammon in Abt's Pediatrics, Philadelphia: Saunders, 1923, 1, 257–444. See also Coppoletta, J. M. and Wolbach, S. B., Am. J. Path., 1933, 9, 55–70. Useful quantitative data on the endocrines are supplied by R. Pearl and his associates in Human Biology, 1935, 7, 350–391, 555–607; 1936, 8, 92–125; 1937, 9, 245–250. For spleen and thymus see Krumbhaar, E. B., Cowdry's Problems of Ageing. Baltimore: Williams & Wilkins, 1942, 139–184. There is a wide range in individual variation. Size may be greater or smaller than the normal or usual without being indicative of disease. Stitt, E. R., Clough, P. W. and M. C., Practical Bacteriology, Haematology and Animal Parasitology. Philadelphia: Blakiston, 1938, 961 pp. give these approximate measurements (abbreviated):

Adrenals—Length, 6–7 cm.; breadth, 3–3.5 cm.; weight, 5–6 gms. each.

Aorta—Length, 42.5–50 cm.; thickness of wall, 1.5–2 mm.; diameter, 1.7–3 cm.; weight, 35–45 gms.

Appendix—Length, 9–10 cm.; diameter, 6 mm.; weight, 7–14 gm., quite variable.

Bladder—Capacity, 500 cc. when normally distended; thickness of wall, 2.5 mm.; weight, 30–60 grams.

Brain—Weight, female, 1250–1275 gms.; male, 1365–1450 gms.; length, 16.5 cm.; transverse diameter, 14 cm.; vertical diameter, 12.7 cm.; dimensions in female being 1 cm. less.

Fallopian tubes—Length, 7.6–12.6 cm., the right usually the longer; diameter of lumen averages 2.5 mm.

Gall bladder—Length, 7.5–10 cm.; diameter, 2.5–3 cm.; thickness of wall, 1–2 mm.; capacity, 30–45 cc.

Heart—Weight, female, 250–280 gms., male, 270–360 gms.; length, 11.5–14 cm.; breadth, 7.5–10 cm.; thickness, 5–8 cm.; thickness, wall left ventricle, 9–12 mm., right ventricle, 2.5–3 mm.; circumference, mitral orifice, 10.4–10.9 cm.; circumference, tricuspid orifice, 12–12.7 cm.; circumference, aortic orifice, 7.7–8 cm.; circumference, pulmonary orifice, 8.5–9 cm.

Intestines—Small intestine, length, 6.75 meters, 2/5 jejunum and 3/5 ileum; diameter from 47 mm. in duodenum to 27 mm. at the end of ileum. Large intestine, length, 180–195 cm.; duodenum, length, 26–28.5 cm.

Kidneys—Weight, left, 150 gms., right, 140 gms.; thickness of cortex, 1 cm.; length, 11.5 cm.; breadth, 6.2 cm.; thickness, 3.2 cm.; the left longer and the right thicker.

Liver—Weight, 1440–1680 gms.; greatest transverse diameter, 20–24 cm., greatest antero-posterior diameter, 10–15 cm., vertical diameter, 12.7–15 cm.

Lungs—Weight, combined, 1020–1290 gms.; weight, male, right lung, 680 gms., left lung, 600 gms.; weight, female, right lung, 480 gms., left lung, 420 gms.; length, 26–30 cm.; antero-posterior diameter at base, 17.5–20 cm.; transverse diameter at base, 10–12.7 cm.; right lung is shorter, broader and thicker than the left; dimensions in female average 2.5 cm. less.

Mammary gland—Weight in adult, 150–200 gms.; weight during lactation, 400–900 gms.

Oesophagus—Length, 25–30 cm.; diameter of lumen, 3 cm.; thickness of wall, 8 mm.; weight, 40 gms.

Ovaries—Weight (each), 4–8 gms., length, 3.8 cm.; breadth, 1.9 cm.; thickness, 1.2 cm.

Pancreas—Weight, quite variable, 60–135 gms.; length varies, average 15–20 cm.

Parathyroids—Length, 6–7 mm.; breadth, 3–4 mm.; thickness, 1.5–2 mm.

Pineal gland—Length, 1 cm.; breadth, 5 mm.; thickness, 5 mm.; weight, 0.2 gm.

Pituitary body—Length, 8 mm.; breadth, 1.2 cm.; weight, 0.3–0.6 gm.
Prostate—Weight, 22 gms.; length 3.1–3.8 cm.; breadth, 3.8–4.5 cm.; thickness, 2.5 cm.
Salivary glands—Parotid, weight, 25–30 gms.; sublingual, weight, 2–3 gm.; submaxillary, weight, 8–9 gms.
Seminal vesicles—Length, 5 cm.
Spinal cord—Length, 45 cm.; weight, 27–30 gms.; transverse diameter averages 1.2 cm.; antero-posterior diameter averages 9 mm.
Spleen—Weight, 155–195 gms.; length, 10–12.5 cm.; breadth, 7.7 cm.; thickness, 2.5–3.7 cm.
Stomach—Capacity, 1–2 liters.; thickness of wall, 6 mm.; weight, 125–175 gms.
Testes—Weight, 20–25 gms. each; length, 3.8 cm.; breadth, 2.5 cm.; thickness, 2 cm.
Thoracic duct—Length, 37–5–45 cm.
Thymus gland—Weight at birth, 13.7 gms. and increases to 26.2 gms. at end of second year when it gradually decreases until gland disappears; dimensions at birth, length, 6 cm.; breadth, 3.7 cm.; thickness, 6 mm.
Thyroid—Transverse diameter, 6–7 cm.; height, 3 cm.; weight, 30–40 gm.
Ureters—Length, 28–30 cm., slightly longer on left side and longer in male, diameter of lumen varies, averages 2.5 mm.
Urethra—Male, length, 16–20.6 cm.; prostatic, 2.5–3.1 cm., membranous, 1.5–2.5 cm., and the anterior, 12–15 cm.; female, length, 3.8 cm.; diameter of lumen averages 7–10 mm.
Uterus—(Virginal) length, 7 cm.; breadth, 4 cm.; thickness, 2.5 cm.; weight, 40–50 gm.; the dimensions of a multiparous uterus are each increased 1 cm. or more and the weight is increased 20 gms.; length of cavity in virgin, 5 cm., in multiparae, 5.7 cm.
Vagina—Length, 7.6–8.9 cm.; posterior wall is slightly longer than the anterior.

**Normals, Microscopical.** Most tissues are examined in but a cursory way. If something is encountered which looks definitely unusual the question of normality comes up, but there are probably numerous instances of tissues which look enough like what was expected to be passed without comment even though they were not in fact normal. This will continue to be the case for appearances that cannot easily be expressed quantitatively. To be specific, the normal range in size of the nuclei of human liver cells is not known, neither are the limits of normal variation in amount of interstitial cells of the testicle appreciated. One difficulty is that a microscopically complete examination of any tissue is very rarely made so there is always a chance that the unseen part deviates from the normal. Particularly is this so in large organs like the liver and lungs and in small ones which characteristically are prone to exhibit regional diversity such as the prostate, thyroid and the mammary glands. Data concerning the gross examination of organs and tissues studied in sections are always desirable and may provide a significant clue. One must not be led astray by histological **Artifacts** or **Postmortem** changes. In experimental animals the problem is less complicated, because the tissues can always be obtained fresh and it is easier to prepare an adequate series of controls for comparison with the suspected specimen. But when we get away from sections to body fluids that can be readily and accurately sampled and in which the cells can be counted per c. mm. both absolutely and differentially the verdict of normal or abnormal can be returned with greater assurance. This is particularly true for the blood and cerebrospinal fluid. Histological criteria of normality are also of some value in the examination of joint fluids, serous fluids and vaginal smears.

**Normoblasts** (orthochromatic erythroblasts). Stage in formation of erythrocyte between erythroblast and reticulocyte; nucleus spherical or oval, picnotic, often excentrically placed. Cytoplasm contains much hemoglobin, not normally present in circulation. See **Erythrocytes,** Developmental series.

**Nucleal Reaction** is a microchemical test for **Thymonucleic Acid** which see, also **Feulgen Reaction.**

**Nuclear Inclusions** are characteristic of some virus diseases but in many such diseases they are not found. Only when they are present in large numbers as in yellow fever is it feasible to investigate them in fresh tissues. Staining reactions, solubility tests and other properties of fresh inclusions are described by Cowdry, E. V. and Kitchen, S. F., Am. J. Hygiene, 1930, **11**, 227–299. Methods for their identification in fixed tissues are summarized by Cowdry, E. V., Am. J. Clin. Path., 1940, **10**, 133–148. For general purposes fixation in Zenker's fluid, paraffin imbedding and coloration with **Hematoxylin** and **Eosin** is the most satisfactory. Coloration with **Phloxine** or **Eosin Methylene blue** gives more brilliant colors but they fade more rapidly. The nuclear inclusions are typically acidophilic and therefore

take eosin and phloxine energetically. When it is desired to reverse the colors use **Safranin-Light Green** which gives green inclusions and red chromatin. For microchemical methods see Cowdry, E. V., Science, 1928, 68, 40–41, see also **Specific Gravity** determinations. Paper by Lucas, A. M., Am. J. Path., 1940, 16, 739–760.

When the following features are noted in a section it is likely that a virus has been at work:

1. A considerable number of inclusion-laden nuclei which can be arranged in series representing stages in development. This indicates an active process in which the nuclei exhibiting the most advanced alterations were affected first and the others in succession.

2. A change in which the accumulation of acidophilic material, forming the inclusion, is accompanied by margination of basophilic chromatin on the nuclear membrane, a disappearance of nucleoli and ultimate death and disintegration of the cells. This suggests that the inclusion formation is not merely an intranuclear heaping up of material effected without injury.

3. A cellular reaction characterized by hyperplasia, hypertrophy or necrosis.

Nuclear inclusions are of two general sorts—A and B (Cowdry, E. V., Arch. Path., 1934, 18, 527–542). Type A are the most definite and exhibit the properties noted above under 2. When the basophilic chromatin does not marginate on the nuclear membrane and the nuclear structure does not disintegrate —we have to proceed warily. Such inclusions (type B) are droplet-like masses of acidophilic material surrounded by clear halos. They have been reported in Borna disease, in poliomyelitis and in several other conditions. When observed in routine preparations they are seldom conspicuous structures. It is only when strongly stained with fuchsin, for instance, that they catch the eye. Perhaps careful search of tissues not subjected to virus action might reveal similar bodies. Therefore in the case of type B inclusions, insistence on criteria 1 and 3 is desirable. The nuclear inclusions in the liver following severe burns look very much like those caused by viruses (Belt, T. H., J. Path. and Bact., 1939, 48, 493–498).

**Nuclease.** This enzyme acting on nucleins is very elusive. A. Van Herwerden has described it in several publications, of which the most recent is Anat. Anz., 1914, 47, 312–325. Lison (p. 175) refers to two other papers by Sachs and Oes but does not give references to them. If one could rely on digestion of sections for two days at 37°C. removing all nucleins to the exclusion of all other cellular materials an important path for investigation would be opened up. A purified nuclease is required. See Barnes, J. M., Brit. J. Exp. Path., 1940, 21, 264–275 for analysis of nuclease in lymphocytes and polymorphonuclear leucocytes.

**Nuclei.** To look into the body and study the nuclei of living cells is feasible only up to a certain point. The observation of the Clarks' (E. R. and E. L., Am. J. Anat., 1936, 59, 123–173) that in transparent chambers inserted into the ears of rabbits (**Sandison's Technique**) the finely granular leucocytes may be followed about and seen to lose their nuclear polymorphism is significant of what can be done. In **Tissue Cultures** the cells are living under less natural conditions but they grow in thin films and can therefore be observed at high magnification. Careful analysis of moving pictures, showing nuclear form and structure, like those of Dr. W. H. Lewis distributed by the Wistar Institute, can prove very fruitful. By ultracentrifugation data can be obtained bearing on intranuclear **Viscosity** and the relative **Specific Gravity** of nuclear components. The techniques of **Microdissection** and microinjection also offer opportunities for advance. The **Vital Staining** of nuclei without killing the cells is difficult and not particularly helpful; but it appears to be feasible in a variety of vertebrate cells with dilute solutions of methylene blue (Russel, D. G., J. Exp. Med., 1914, 20, 545–553), in amebae by microinjection (Monne, L., Proc. Soc. Exp. Biol. R Med., 1934–35, 32, 1197–1199), and in the fibroblasts of tissue cultures with crystal violet (Bank, O. and Kleinzeller, H., Arch. f. exp. Zellf., 1938, 21, 394–399). The same can be said for **Ultraviolet Photomicrography.**

The choice of fixative is important. It is difficult to secure after formalin fixation a brilliant color contrast of basophilic and acidophilic nuclear materials by staining with **Giemsa, Eosin-Methylene Blue** and other mixtures of "basic" and "acid" dyes, because the former take very intensely and the latter, lightly. But following Zenker's fluid and other mixtures containing potassium bichromate, which acts as a sort of mordant, these stains color the acidophilic as well as the basophilic components. It is for this reason, and because nuclear inclusions caused by virus action are usually acidophilic,

that formalin used alone is contraindicated as a fixative.

On the morphological side it is known that nuclei stained in sections after fixation in the usual ways show a diversity, or heterogenicity, of internal structure which cannot be observed by the most careful examination of the nuclei of living cells. In the latter only the nucleolus can generally be distinguished. The so-called linin network, and small irregular particles staining with acid and basic dyes, are not observed. These probably result from the coagulating action of the fixative upon materials present in solution or fairly uniformly distributed in the nucleoplasm. Stained sections of tissues fixed in fluids containing fair amounts of osmic acid (**Altmann's Mixture** and **Bensley's Acetic-Osmic-Bichromate**) exhibit, on the contrary, nuclei with quite homogeneous looking nucleoplasm, containing nucleoli, which portray the condition *in vivo* more accurately. Colored illustrations of the nuclei of liver cells containing inclusions after osmic and non-osmic fixation (Figs. 47 and 20) are provided by Cowdry, E. V. and Kitchen, S. F., Am. J. Hyg., 1930, **11**, 227–299. This does not mean, however, that the ground substance is always optically homogeneous *in vivo*.

The shrinkage of nuclei when examined in stained sections is generally more than 10% of their size *in vivo*. In post-mortem autolysis, particularly of the kidney, one of the first nuclear modifications is shrinkage. The shrunken nuclei may stain intensely with both basic and acid dyes. The acidophilic material in them may even appear to be increased; for it is more concentrated, owing to decrease in volume (oxychromatic degeneration). They are also more spherical and less oval in shape. In early stages this modification can easily be identified by its occurrence in some tubules and not in others. A comparable hyperchromatism of nuclei at the edge of a section accompanied by a flattening of them may indicate that a surface film of tissue was permitted to dry before fixation.

Among the stains **Iron Hematoxylin** is a favourite because of its sharpness and permanence. **Phloxin-Methylene Blue** is also recommended. If one desires to reverse the colors and get red nuclei and green cytoplasm **Safranin Light Green** is suggested. The **Safranin-Gentian Violet-Orange G** technique gives several beautiful color tones. Recently the Feulgen reaction by which **Thymonucleic Acid** can be demonstrated has become very popular as the most sharply discriminating nuclear stain. Microchemical studies are now possible which a few years ago were undreamed of. The method of **Microincineration** reveals some of the mineral constituents (Scott, G. H., Proc. Soc. Exp. Biol. & Med., 1935, **32**, 1428–1429).

The collection of nuclei in bulk for chemical analysis is now feasible (see **Centrifugation**). Thus nuclei of liver cells can be separated from cytoplasms by centrifugation after treatment with dilute citric acid. Normal liver nuclei do not accumulate $P_{32}$ while tumor nuclei and regenerating nuclei do (Marshak, A., Federation Proceedings, Baltimore, 1942, **1**, (2) 57). A method for separating nuclei from rest of thymus is described by Williamson, M.B and Gulick, A., J. Cell. & Comp. Physiol., 1942, **20**, 116–118. The authors analysed the mass of nuclei for calcium, magnesium and phosphorus. Another method for separating from cytoplasm (Crossmon, G., Science, 1937, **85**, 250) is to place drop 5% aq. citric acid in center of a slide smeared with Mayer's **Albumin Glycerin.** Add piece fresh muscle. This slowly becomes transparent and infiltrated. The cloudiness of the citric acid is caused by released nuclei. Remove muscle and allow fluid containing nuclei to dry completely. Hold nuclei in place by treating with 95% ethyl alcohol. Wash in tap water, then in aq. dest., stain with Mayer's **Hemalum,** blue in tap water, counterstain in eosin, dehydrate, clear and mount. Perhaps the technique can be so adjusted that it will permit the separation of nuclei from other tissues. See **Arginase.**

**Nucleocytoplasmic Ratio.** A histological method for computing this ratio is fully described by Cowdry, E. V. and Paletta, F. X., J. Nat. Cancer Inst., 1941, **1**, 745–759; but there are many such techniques. A chemical method has been used to advantage by Dawbarn, M. C., Australian J. Exp. Biol. & Med. Sci., 1932, **9**, 213–226. Her ratio is obtained by dividing the nucleic acid nitrogen by the total coagulable nitrogen less nucleic acid nitrogen.

**Nucleolinus** is a term introduced by Haeckel to indicate a deeply staining granule within a nucleolus. For details see Champy, C. and Carleton, H.M., Quart. J. Micr. Sci., 1921, **65**, 589–610.

**Nucleolus** (L. dim. of nucleus) is a body within a nucleus. There are at least three sorts.

1. *Plasmosomes.* These can be defined as roughly spherical bodies, which

can easily be seen in the nuclei of some living cells without the aid of any stains, which stain after appropriate fixation, namely, with plasma or "acid" stains like eosin, (hence the name) and which do not directly contribute material to the formation of chromosomes.

Plasmosomes are not to be confused with cytoplasmic granules called plasmosomes by Arnold many years ago or with plastosomes, a term given by Meves to mitochondria and now fortunately being discarded. They can be referred to as acidophilic or oxyphilic nucleoli, but sometimes they are tinged quite strongly with basic dyes. They are of dense consistency, easily shifted by centrifugal action and are in some cases more resistant to the digestive action of pepsin and hydrochloric acid than karyosomes.

2. *Karyosomes*, are by contrast intensely basophilic and do contribute material to the making of chromosomes during mitosis. But they are resistant to peptic hydrochloric acid digestion. Wilson (E. B., Heredity, New York: Macmillan 1925, p. 93) recognizes 3 types, net-knots, chromosome-nucleoli and karyospheres. There is doubt about the existence *in vivo* of the net-knots in the same shape, size and position as observed in stained sections.

3. *Amphinucleoli* (G. *amphi* on both sides) are nucleoli consisting of both plasmosome and karyosome material. Often the acidophilic substance acts as a kind of core and the basophilic substance is close to it or appears to be plastered on its surface. The latter may not occur in the same form in the living nucleus.

The fixation which shows, when the sections are stained, the highest degree of nucleolar detail is not necessarily the best (see remarks about **Nuclei**,) The **Linin** network, net-knots and basophilic material marginated on plasmosomes may result in part from the coagulating action of the fixative on material originally distributed diffusely in the nucleoplasm. Nucleoli which look bubbly, or are surrounded by halos, are to be regarded with suspicion. Fixation in **Acetic-Osmic-Bichromate** and in other fluids containing osmic acid is indicated but they penetrate poorly. Staining by almost any technique which gives a good color contrast between acidophilic and basophilic materials is satisfactory. The classical stain is with safranin and light green. Eosin and methylene blue, hematoxylin and eosin are recommended, likewise all methods advised for **Nuclear Inclusions** caused by viruses.

Usually no difficulty is experienced in the identification of nucleoli. However with the plasmosomes there may be some question. In the first place nuclear inclusions type B (Cowdry E. V., Arch. Path., 1934, 18, 527–542) look something like plasmosomes. For example, the nuclear inclusions in Borna disease are acidophilic and may be of the same size as plasmosomes; but, they like others of type B are strongly acidophilic, are seldom tinged with basic stains and are generally surrounded by halos of unstained nucleoplasm. Moreover they are not present in normal animals.

Secondly cells are sometimes encountered in which there is an increase in acidophilic nuclear material often accompanied by nuclear hypertrophy. The material may occur in the form of dense spherules or of masses which are bluntly angular and without halos. Colored illustrations of liver cell nuclei are given by Cowdry, E. V. and Kitchen, S. F., Am. J. Hyg., 1930, 11, 227–299, figs. 43 and 44. These bodies may be true nucleoli which have undergone hypertrophy or they may be simply accumulations in the nuclei of acidophilic material. The only sure way to tell would be to ascertain whether they comport themselves like true plasmosomes during mitosis but the cells involved have not been seen in division. In other conditions (gliomatous tumors, etc.) cells are found whose nuclei are enlarged and possess roughly spherical, vacuole-like masses of granular acidophilic material. The granules have the appearance of coagula produced by the fixative in a rather thin fluid medium. There is no halo. Such bodies are probably not altered plasmosomes. Their density is much less.

*Differential staining.* Nucleoli are colored brown after fixation in equal parts of 1% aq. chromic acid and 10% formalin and staining of chromosomes by **Feulgen Reaction** (Bhaduri, P. N., J. Roy. Micr. Sci., 1938, 58, 120–124).

**Nucleonucleolar Ratio** recommended as an aid in the grading of malignancy with review of the literature (Mendes Ferreira, H. E., J. Lab. R Clin. Med., 1940–41, 26, 1612–1628).

**Nucleotides,** see **Pentose Nucleotides.**

**Nutriles,** growth promoting (Williams, R. J., Biol. Rev., 1941, 16, 49–80).

**Oil Red IV,** see **Sudan IV.**

**Oil Red AS, O, B** or **3B,** see **Sudan III.**

**Oil Red O** (CI, 73).—fast oil orange II, fat ponceau, oil scarlet, orange RR, red B, Sudan II—an acid mono-azo dye suggested as fat stain by French, R. W.,

Stain Techn., 1926, **1**, 79. Proescher's (F., Stain Techn., 1927, **2**, 60–61) oil red pyridine stain for fat is to immerse frozen sections of formalin, Muller-formalin (see Muller's fluid) and 5 cc. 10% formalin in 100 cc. sat. aq. picric acid fixed tissues in 50% aq. pyridine, 3–5 min. Stain 3–5 min in 3–5 gms. oil red O dissolved in 100 cc. 70% aq. pyridine C.P. Differentiate in 50% pyridine several minutes and counterstain for 2–3 min. in **Delafield's Hematoxylin.** Mount in levulose syrup. For central nervous system differentiate 30 min. in pyridine and use 16 cc. Delafield's + 2 cc. glacial acetic acid. According to Proescher, oil red O stains fats and lipids more intensely and quickly than Sudan III or IV.

**Oil Scarlet,** see **Oil Red O.**

**Oil Vermillion,** see **Sudan R.**

**Okajima's** "omnichrom" stain (Ito, T., Folia Anat. Jap., 1937, **15**, 357–359).

**O'Leary's Brazilin Method.** For myelin sheaths. Run paraffin, or celloidin sections of properly fixed and mordanted (**Muller's Fluid**) tissue to water. Immerse in 3% aq. potassium bichromate or in Muller's fluid, 12–24 hrs. Stain in: 10% Grubler's Brazilin in abs. alc. (1–6 months old), 10 cc.; aq. dest., 100 cc.; acetic acid, glacial, 5 drops. Wash in aq. dest. Differentiate in 0.25% aq. potassium permanganate 1–5 min. Remove potassium permanganate with Weil's solution (oxalic acid, 2.5 gm.; sodium bisulphite, 2.5 gm.; aq. dest. 1,000 cc.) Sections should show gray matter light pink, white matter brilliant red. Cell bodies stain in addition to myelinated fibers. If differentiation not complete after first immersion in potassium permanganate followed by oxalic acid-bisulphite mixture, repeat the procedure. Wash, dehydrate and mount.

**Oligodendroglia.** Method for impregnation with silver in pyroxylin (celloidin) sections (Weil, H. and Davenport, H. A., Trans. Chicago Path. Soc., 1933, **14**, 95–96). This resembles their **Microglia** method. Wash sections in aq. dest. and transfer to aq. dest. containing 1 drop conc. ammonia per 10 cc. Treat for 15–20 sec. with silver solution made up as for microglia except that 15% aq. silver nitrate is used and the end point of the titration is reached when about 12 cc. of it have been added to the 2 cc. conc. ammonia. Transfer to 10% formalin and allow section to drop to bottom without moving dish. After the pyroxylin has become deeply stained and the tissue begins to take a brown color, move it with glass rods until it is stained coffee-brown. Use fresh formalin for each section. Pass section through 3 changes aq. dest. Dehydrate in alcohol, clear in xylol and mount in balsam.

**Olive Oil,** reactions in tissue to fat stains after various fixations (Black, C. E., J. Lab. & Clin. Med., 1937–38, **23**, 1027–1036).

**Omentum,** spreads of (McClung, p. 336). Transplants of spleen into (Holyoke, E. H., Am. J. Anat., 1940, **66**, 87–132.

**Opsonocytophagic Index,** method for rapid staining of blood smears in (Bondi, A. Jr., J. Lab. & Clin. Med., 1941, **26**, 1811). Derivation of *index number* in (Foshay, L., LeBlanc, T. J., J. Lab. & Clin. Med., 1936–37, **22**, 1297–1300).

**Optic Lens,** methods of microincineration and histospectrography as applied to cataracts of various sorts and normal lenses with special attention to copper, zinc and iron (Busnel, R. G., Pillet, P. and Tille, H., Bull. d'Hist. Appl., 1938, **15**, 99–109).

**Oral Mucosa.** Smear method for study of keratinization (Weinmann, J., J. Dent. Res., 1940, **19**, 57–71). With end of agate spatula gently scrape area about 1.5 sq. cm. Smear on slide, dry in air and stain for 30 sec. in: sat. alc. gentian violet (or better crystal violet) 10 cc. + 5% aq. phenol, 90 cc. **Lugol's Iodine,** 30 sec. Wash in water until no more color is extracted. Counterstain for 2 min. in sat. safranin O in 95% alcohol, 10 cc. + aq. dest., 100 cc. Wash in water 2–3 sec., dry and mount in balsam.

**Orange I** (CI, 150). Synonyms: naphthol orange, tropaeolin G or 000 No. 1. An acid mono-azo dye used as an **Indicator.**

**Orange II** (CI, 151). Synonyms: acid orange II, Y or A, gold orange, mandarin G, orange A, P, or R, orange extra, tropaeolin 000 No. 2. An acid mono-azo dye. Ebbinghaus, H., Centralbl. f. allg. Path. u. Path. Anat., 1902, **13**, 422–425 employed gold orange with hematoxylin as a special stain for keratin.

**Orange III,** see **Methyl Orange.**

**Orange A, P,** or **R,** see **Orange II.**

**Orange Extra,** see **Orange II.**

**Orange G** (CI, 27). Synonym; wool orange 2G. Of slightly different grade according to Conn (p. 47) are orange GG and GMP. An acid mono-azo dye widely used.

**Orange MNO** or **MN,** see **Metanil Yellow.**

**Orange RR,** see **Oil Red O.**

**Orcein** (CI, 1242) is a natural dye produced from *lecanora parella* (a lichen) and should not be confused with *orcin* produced from the same plant. It is now prepared synthetically. Its precise formula remains to be determined but it is a most valuable stain for **Elastic Fibers.** Mollier, G., Zeit. f. wis. mikr.,

1938, **55**, 472–473 employed it with iron hematoxylin, naphthol green B and azocarmine G. Acetic-orcein is advocated as a new stain-fixative for chromosomes (LaCour, L., Stain Techn., 1941 **16**, 169–174).

**Origanum Oil.** With it tissues can be cleared from 95% alcohol, but care must be taken to obtain a pure product. The kind required consists of carvacrol and cymene terpenes. Ordinary origanum oil is oil of thyme.

**Orseillin BB** (CI, 284). A little used acid dis-azo dye. See Cohen, I., and Doak, K. D., Stain Techn., 1935, **10**, 25–32. For staining fungi (Alcorn, G. D. and Yeager, C. C., Stain Techn., 1937, **12**, 157–158).

**Orthochromatic Erythroblasts,** see **Erythrocytes,** developmental series.

**Orth's Fluid.** Potassium bichromate, 2.5 gm.; aq. dest., 100 cc., formalin, 10 cc. The 1 gm. sodium sulphate originally advised by Orth is omitted as useless. Since the fluid does not keep it should be made up immediately before use. Regaud's fluid, the best fixative for mitochondria, is the same except that the amount of formalin is increased See **Lithium Carmine** (Orth).

**Osmic Acid.** This is the tetroxide of osmium and has no acid properties. It comes in sealed glass tubes usually each containing 1 gm. To make the 2% aq. sol. of osmic acid generally employed, wash the label off the tube with soap and water. After washing repeatedly in aq. dest. rinse in absolute alcohol and dry. Carefully clean the inside of a glass stoppered bottle and of a graduate in the same way. With clean forceps put the tube in the bottle. If it is not easily broken by vigorous shaking it will be necessary to take it out, file one side, break and return to the bottle. Finally add 50 cc. of aq. dest, measured in the graduate. The osmic acid slowly dissolves forming a clear light yellow solution. Do not hasten solution by heat. Keep in dark or subdued light. To use a bottle made of colored glass or the outside of which has been blackened is a bad practice because it hides the condition of the solution from the person using it. If there is a blackening of the solution its potency is probably reduced. An indicator of concentration, discovered by Tschngaeff, has been improved by Palmer (R., J. Roy. Micr. Sci., 1930, **50**, 221–226).

The fumes of osmic acid are very injurious to the eyes. They are a good fixative for well separated cells as in smears. They blacken the chromaffin cells of the adrenal charged with epinephrine or its precursor (Cramer, W., Fever, Heat Regulation, Climate and Thyroid-Adrenal Apparatus. London: Longmans, Green & Co., 1928, 153 pp.) Alone, a solution of osmic acid is a fair fixative for mitochondria and by prolonged action may reveal the **Golgi apparatus.** See critique by Owens and Bensley (H. S. and R. R., Am. J. Anat., 1929, **44**, 79–109). But osmic acid penetrates very badly indeed and is best employed in mixtures with other chemicals as in the fixatives of **Altmann, Mann, Bensley, Flemming** and others. Its chief value is that it blackens many but not all fatty droplets. However it also blackens some materials which are not fatty. Osmic acid plays an important part in the **Marchi method** for nerve fiber degeneration.

**Osmic Acid Method** for fat. When reduced to osmium dioxide in the presence of some fats it blackens them as may be seen by the examination of tissues fixed in fluids containing osmic acid (Altmann's, Flemming's etc.) but unless rigidly controlled other substances may be blackened as well or not all of the fats may be shown. See remarks by Owens, H. B. and Bensley, R. R., Anat. Rec., 1929, **44**, 79–109. It is best to proceed as advised by Mallory (p. 119). Place frozen sections of tissue fixed in 10% formalin for 24 hrs. in aq. dest. 1% osmic acid 24 hrs. (or Flemming's or Marchi's solution). Wash thoroughly in running water 6–12 hrs. Abs. alc. for several hours in order to get secondary staining of palmitic and stearic compounds as well as of oleic. Wash in water and mount in glycerin jelly (glycerin alone will do). Fat is black against a yellowish brown background. Non-fatty substances like tannic acid and eleidin of epidermis are also blackened.

For nerve fibers (Dr. J. L. O'Leary, personal communication). Use fresh or 10% formalin fixed material. Tie a stretch of freshly isolated nerve to short length of glass rod and immerse in 2% aq. osmic acid. Leave for 24 hrs. Wash 4–6 hrs. in running water. Dehydrate in ascending alcohols and doubly imbed by the **Peterfi** method as follows: Pour 1% celloidin in methyl benzoate (which takes about 1 month to dissolve) into a dish. Add absolute alcohol and the tissue. The latter gradually sinks into the celloidin. Transfer to 2–3% celloidin in methyl benzoate. Leave 2–4 days. Drop tissue directly into benzol. After a few hours in benzol begin infiltration in paraffin at 40°C. This takes 12–24 hrs. Change paraffin several times and imbed.

**Ossification.** Demonstration of in embryos and fetuses up to 18 weeks by staining

with alizarin red S (Richmond, G. W. and Bennett, L., Stain Techn., 1938, **13**, 77–79). Eviscerate. Fix in 95% alcohol 2 weeks or more. Rinse in tap water and put in 1% aq. $K_2CO_3$ for month or longer. Clear soft parts and make bones clearly visible by placing in 1% aq. KOH for 10 days or more. (Specimens fixed in formalin instead of alcohol require about 1 month in 10% KOH) If tissues become too soft harden in equal parts glycerin, 95% alcohol and water 12–24 hrs. and continue KOH if necessary. In last few days reduce KOH to 0.5%. Wash in running tap water 12 hrs. Immerse in 0.1% aq. alizarin red S to which few drops 1% aq. KOH has been added for 30–60 min. Wash for 30 min. in running tap water. Remove deep purple color from soft parts by immersing in 20% aq. glycerin containing 1% KOH. For small specimens reduce KOH to 0.5%. This decolorization may require 1–2 weeks before ossified skeleton remains deep red in transparent background. Dehydrate by passing slowly through 95% alc., glycerin and aq. dest. in following proportions 10:20:70—20:20:60—30:30:40—40:40:20—50:50:0. Seal in specimen jar in the final mixture of alcohol and glycerin.

A rather similar technique leading up to dehydration in absolute alcohol, clearing in toluol and final storage in anise oil saturated with naphthalene is presented by Cumley, R. W., Crow, J. F. and Griffen, A. B., Stain Techn., **14**, 7–11. This staining of ossification centers with alizarin red can be combined with the coloration of the cartilaginous skeleton with toluidin blue to make quite brilliant specimens (Williams, T. W., Stain Techn., 1941, **16**, 23–25).

**Ossification,** intensive glycogenesis during (Gendre, H., Bull. d'Hist. Appl., 1938, **15**, 165–178).

**Otoliths,** technique for (Johnston, M., J. Roy. Micr. Soc., 1938, **58**, 112–119).

**Ova,** concentration of parasitic ova in **Feces.**

**Ovary.** For routine purposes fixation in **Zenker's Fluid** and coloration by **Mallory's Connective Tissue** stain or by **Masson's Trichrome** technique is indicated. Follicular atresia can be beautifully demonstrated by **Vital Staining** with trypan blue or by other similar dyes, see Evans, H. M. and Swezy, D. R., Memoirs Univ. California, 1931, **9**, 119–224. For the utilization of **Microdissection** in determination of the physical properties of the follicular wall see Thanhoffer, L., Zeit. f. Anat. u. Entw., 1933, **100**, 559–562. The interesting fluorescence studies on the ovary by Policard, A., C. rend. Acad. d. Sci., 1924, **179**, 1287 are likely to be extended now that the possibilities of **Fluorescence Microscopy** are better appreciated.

**Owen's Blue** (British Drug Houses Ltd.), a dis-azo dye similar in composition to Manchester blue. Used best in alcoholic solution (H. G. Cannan, J. Roy. Micr. Soc., 1941, **61**, 88–94).

**Oxalate Solutions,** see **Anticoagulant Solutions.**

**Oxazins.** Dyes resembling the thiazins but in which sulphur atom is replaced by oxygen. Examples: brilliant cresyl blue, celestin blue B, cresyl violet, gallamin blue, gallocyanin, Nile blue sulphate, resorcin blue.

**Oxidase.** Unfortunately, as Lison (p. 263) points out, histologists and biochemists are not always agreed as to terms. The latter include under the designation "oxidases" all enzymes capable of catalysing a reaction of oxidation, for instance the phenolases, purinoxidases, succinoxidase, tyrosinase, etc.; whereas what the former describe as "oxidases" are in reality phenolases and thus only a part of the whole group of oxidases. The action of oxidase (or phenolase) in the presence of $O_2$ is the same as a peroxidase in the presence of $H_2O_2$. But the particular oxidases are more delicate and easily modified in their action by variations in temperature, pH and other factors. The following methods are from Lison, much abbreviated.

1. M. nadi oxidase reaction (Gräff) = oxidase reaction, modification A (W. H. Schultze) and stabile oxidase reaction (V. Gierke). Make 2 solutions: A. Boil 1 gm. $\alpha$ naphthol in 100 cc. aq. dest. Add drop by drop 25% aq. potassium hydroxide until melted $\alpha$ naphthol is dissolved. Cool. Can be kept in dark at least 1 month. B. Obtain good sample dimethyl - p - phenylenediamine furnished in sealed tubes. It blackens quickly when secured in bulk. Gräff advised, as more stable, dimethyl-p-phenylenediamine hydrochloride. Make 1% solution of either in aq. dest. Boil and cool. Keeps 2–3 weeks in dark. Immediately before using take equal parts A and B, filter and employ filtrate. Place frozen sections of formalin fixed tissues or smears (after fixing for 2 hrs. in formalin vapor or in formol, 10 cc. + 96% alcohol, 40 cc.) in above mixture of A and B in a thin layer at the bottom of a Petri dish. Agitate a little to permit oxygenation of the fluid. Blue granules quickly appear (1–5 min.). Rinse in water and examine. To make more permanent treat with Lugol's iodine diluted one third, 2–3 min.,

which makes the blue granules brown. Restore blue by washing in aq. dest. + few drops sat. aq. lithium carbonate. Counterstain with hemalum or safranin, mount in glycerin. Schmorl advised instead of Lugol's a conc. aq. sol. ammonium molybdate.

2. G. nadi oxidase reaction (Gräff) = labile oxidase reaction (V. Gierke). This more difficult method is for fresh tissues. The nadi reagent is prepared without addition of alkali. The required pH depends on the cells investigated. For animal tissues Lison recommends about 8.2, 8.1 and 7.8 and for plants 3.4–5.9. Directions are given by Gräff (S., Die Mikromorphologischen Methoden der Fermentforschung, Abderhalden's Handb., 1936, 4 (1), 93–142).

3. Naphthol reaction of Loele. This is not, in the opinion of Lison, strictly speaking a microchemical reaction, but it is as simple. Place small amount $\alpha$ naphthol in a test tube. Add drop by drop 10% aq. potassium hydroxide until naphthol is completely dissolved. Add 200 cc. aq. dest. Solution may be used after 24 hrs. It will last about 3 weeks. Frozen sections of formalin fixed tissues treated with this reagent show violet or black granules, which quickly disappear.

**Oxidation-Reduction Potential.** Details supplied by Dr. Christopher Carruthers of The Barnard Free Skin and Cancer Hospital.

This very important measurement is particularly well explained by Seifriz, W., Protoplasm, New York: McGraw-Hill Book Co., 1936, 584 pp. For a comprehensive developmental treatment of the subject see Clark, W. M. and coworkers, Hygienic Laboratory Bull., 1928, **151**, 1–352.

Oxidation is the process in which a substance loses electrons, and reduction is the process in which a substance takes on electrons. For example when ferric chloride $FeCl_3$ gains an electron it is reduced to $FeCl_2$, or

$$Fe^{+++} + \text{electron} \rightarrow Fe^{++}$$

Because the ion, $Fe^{++}$, can lose an electron it is a reducing agent or *reductant*, and since $Fe^{+++}$ can gain an electron it is an oxidizing agent or *oxidant*. The change is reversible

$$Fe^{+++} + \text{electron} \rightleftharpoons Fe^{++}.$$

When an acid mixture of ferrous and ferric chloride is placed in an electrode vessel it will yield a potential—the oxidation potential. This potential can be measured by placing a noble metal, such as a bright platinum wire in the solution, and measuring the potential against the normal calomel electrode with a potentiometer. The intensity of the oxidizing or reducing action of a system is determined by its oxidation potential. The potential produced is determined by the ratio of ferrous to ferric ions, and is given by the relation:

$$E_h = E_o - \frac{RT}{F} \ln \frac{(Fe^{++})}{(Fe^{+++})};$$

$$\frac{(\text{Reductant})}{(\text{Oxidant})}.$$

$E_h$ is the observed difference in electromotive force between the electrode and the normal hydrogen electrode; $E_o$ is a constant characteristic for the ferrous-ferric system (the so-called normal potential); $R$, $T$, and $F$ have their customary significances. The parentheses represent concentrations of the two components.

Certain groups of organic dyes are likewise able to induce upon electrodes reversible potentials. These organic dyes can be used as indicators of oxidation-reduction, and the following relation holds:

$$E_h = E_o - \frac{RT}{nF} \ln \frac{(\text{Red})}{(\text{Ox})}$$

If the reductant is identified as an ion, or the oxidant as a cation, for two simple cases there would be

$$\text{Ox} + \text{electron} \rightleftharpoons \text{Red}^- \qquad (1)$$

$$\text{Ox}^+ + \text{electron} \rightleftharpoons \text{Red} \qquad (2)$$

For equation (1), the relation would be

$$E_h = E_o - \frac{RT}{nF} \ln \frac{(\text{Red}^-)}{(\text{Ox})}$$

The active reductant of equation (1) is the anion of an acid, and its concentration depends not only upon the amount of reductant, but also upon the hydrogen ion concentration. The relation then becomes

$$E'_o = E_o - \frac{RT}{nF} \ln \frac{(\text{Red}^-)}{(\text{Ox})}$$

at any constant pH (For development see Cohen, B., Symposia Quant. Biol., 1933, **1**, 195–204).

The use and interpretation of indicator dyes in biological systems is given by Cohen, B., *ibid*, 214–223, and Chambers, R., *ibid*, 205–213. Sources of error are also indicated by Cohen, B., Chambers, R. and Reznikoff, P., J. Gen. Physiol., 1928, **11**, 585–612. Most of the following material is taken from the above papers.

On a microscopic basis, the measurements, like those of pH, are made with indicators in which the cells are bathed

or which are injected with them. They are applied in sequence and their reactions observed. Methylene blue, for instance, will be oxidized (retain color) or be reduced (lose color) depending upon the relative activity of the processes of oxidation and reduction.

Although it is difficult accurately to measure the amount of indicator injected into cells, it is imperative that the quantity be small. Otherwise too much indicator may be more than the cell can reduce, or be greater than the reducing intensity which the cell can generate. The following indicators from Cohen provide a useful range in potential values:

| Name of Oxidant | $E'$ at pH 7.0 |
|---|---|
| Phenol m-sulfonate indo-2,6 dibromo-phenol | 0.273 |
| m-Bromophenol indophenol | 0.248 |
| o-Chlorophenol indophenol | 0.233 |
| Phenol blue chloride | 0.227 |
| Phenol indo-2,6 dichlorophenol | 0.217 |
| o Cresol indophenol | 0.195 |
| o Cresol indo-2,6 dichlorophenol | 0.181 |
| 1-Naphthol-2-sulfonate indophenol o-sulfonate | 0.135 |
| 1-Naphthol-2-sulfonate indophenol | 0.123 |
| Toluylene blue chloride | 0.115 |
| Brilliant cresyl blue chloride | 0.047 |
| Methylene blue chloride | +0.011 |
| $K_4$ indigo tetrasulfonate | −0.046 |
| Ethyl capri blue nitrate | −0.072 |
| $K_3$ indigo trisulphonate | −0.081 |
| $K_2$ indigo disulphonate | −0.125 |
| Cresyl violet | −0.167 |

$E'_0$ represents the potential at any given pH ot a system in which the ratio of oxidant to reductant is unity.

In order to get the indicator dyes into single cells the microinjection technique of Chambers is used. Chambers recommends dilute aqueous solutions of the basic dyes, i.e., 0.05% to 0.1%, and injects successive small doses. Needham, J. and D. M., Proc. Roy. Soc. B, 1926, **99**, 173–199; 383–397 used 1% solution since weaker solutions of particular dyes could not be seen under the microscope when injected into cells.

The determinations are carried out aerobically (cells maintained in a micro drop in water-saturated air at atmospheric pressure) and anaerobically (cells held in an atmosphere of purified process nitrogen saturated with water).

For example, under aerobiosis, if all the indicators down to and including methylene blue are reduced at pH 7.0 by cells of a particular type; and if ethyl capri blue is only partially reduced (and the rest of the indicators not reduced), the reducing intensity of the aerobic cell is approximately −0.072 volts at pH 7.0. The same procedure is followed for cells anaerobically.

To detect the presence of the indicator after decolorization by the cell protoplasm, reoxidation of the reductant can be accomplished by injecting dilute potassium ferricyanide or of potassium dichromate in the anaerobic state, or by exposure to air in the anaerobic state. The recovery of color on oxidation is a necessary control demonstrating that the indicator has been reversibly reduced and not reversibly destroyed.

It is also essential to bring the cell interior into contact both with oxidant and reductant of the indicator. This is necessary to determine whether the indicator, which would shift to the potential of the electromotive system present, is behaving in a truly reversible manner.

The aqueous solutions of the acid dyes, e.g. the various indophenols give the most clear cut results. Upon injection they rapidly diffuse throughout the cell before being reduced. The experimental evidence indicates that the speed of reduction of the indicator dyes decreases as the potential of the indicator approaches that of the cell.

In the immersion method slices of tissue are bathed in solutions of the indicator dyes. Here it is not only necessary to distinguish between penetrating and nonpenetrating indicators but also to watch for differences in the rapidity with which cells and certain cell inclusions are stained by the various indicators. For example, indicators containing the sulfonated radicals do not readily penetrate cells, while the nonsulfonated more or less rapidly penetrate.

Fildes, P., Brit. J. Exp. Path., 1929, **10**, 151–175 measured the oxidation-reduction potential of the subcutaneous tissue fluid of the guinea pig, and also its effect on infection. Guinea pigs were inoculated with indicator dyes (0.01%) in both the reduced and oxidized states and he observed whether change had occurred. The injections were made superficially so that the immediate effect could be seen through the shaved skin. The oxidized form of methylene blue remained a strong blue, and the reduced dye assumed a distinct blue color. This indicated that the subcutaneous tissue maintained an oxidation-reduction potential on the positive side of reduced methylene blue.

Then "indophenol 1" (naphthol-2 sodium sulphonate indo 2, 6 dibromophenol) in both states was injected and the animals examined. After 40 min-

utes the oxidized and reduced forms of the dye were at about the same intensity of blue. Therefore it was concluded that the $E_h^+$ of the subcutaneous tissue was positive to that of reduced indophenol 1. The rate of oxidation was slower here than in the case of methylene blue, because the difference in $E_h$ of the tissues and the reduction point of the dye was less.

$$E_h = E_o' - 0.062 \log \frac{a}{100 - a} \quad \text{(at 37°C.)}$$

where $E_o'$ is a constant characteristic of the particular system and $a$ = % reduction.

Finally the dye indicator, "indophenol 2" (phenolindophenol 2, 6 dibromophenol) was injected. The reduced form of the dye remained colorless while the oxidized form faded from 20 to 80 minutes. Addition of ferricyanide failed to restore all the reduced dye, so the results were complicated by decomposition of the dye in the tissues. It was concluded that the $E_h$ of the tissue fluids is positive to the zone of complete reduction of indophenol 1.

The oxidation-reduction potential of the ciliary body was determined (Friedenwald, J. S. and Stieher, R. D., Arch. Ophth., 1938, **20**, 761–786) by introducing indicator dyes into the stroma or epithelium of ciliary body under aerobic and anaerobic conditions. After equilibrium had been reached, the degree of bleaching was observed microscopically. Then an oxidizing agent was added, such as ferricyanide, and recovery of the color was noted. The ratio of intensity of color before and after oxidation with ferricyanide gave the potential in the system since it afforded a measure of the ratio of oxidant to reductant of the indicator in equilibrium in the tissue. Aerobically the epithelium had an estimated potential of +0.100 volts, and the stroma −0.130 volts. Anaerobically both had estimated potentials of −0.290 volts.

Lewis, M. R., Barron, E. S. G. and Gardner, R. E., Proc. Soc. Exp. Biol. & Med., 1930–31, **28**, 684–685 compared the power of cancer tumors, tumors produced by viruses and normal tissue to reduce methylene blue. The tissues were cut in a manner similar for tissue respiration, and the pieces were placed in M/15 Sorensen's phosphate buffers at pH 7.38. Anaerobiosis was maintained by a stream of nitrogen. The time of reduction of the dye by tumors was the same as that of normal tissues.

Voegtlin, C., Johnson, J. M. and Dyer, H. A., J. Pharm. & Exp. Therap., 1925, **24**, 305–337 have quantitatively estimated the reducing power of normal and cancerous tissue. For the anaerobic experiments tissues were sliced about 2 mm. thick and weighed about 0.5 gm. Samples of tissue were placed in sterile vacuum tubes, and 5 cc. of a sterile solution of the indicator in a phosphate buffer solution (M/15 $Na_2HPO_4$, $KH_2PO_4$ Sorensen) of pH 7.6 were added to each tube by means of a sterile pipette. After evacuation of the tubes by a vacuum pump, they were rapidly fixed in a constant temperature bath at 38°C. on a revolving rack.

The indicator solutions were prepared by adding phosphate buffer to an accurately weighed amount of the dye in a mortar and grinding. The solutions were made up to volume and boiled to sterilize.

The reducing power of tissues was based upon the time needed to reduce anaerobically equimolar amounts of the indicators used (the dye content of each indicator was determined on a moisture free basis). For the indicators used it was found that the optimum concentration for comparative purposes was approximately M/42,533. A more useful concentration of M/40,000 was suggested for future work.

All the tissues (brain, carcinoma—peripheral portion, heart muscle, spleen, kidney, liver, lung, skeletal muscle and testis) had a reducing power which varied according to the type of tissue having the highest reducing power (with the exception of the necrotic portion of carcinoma). The latter was devoid of reducing power while the viable portion reduced the indicators as rapidly as did some of the normal tissues.

**Oxychromatic Degeneration.** A kind of degeneration in which oxychromatic (acidophilic) material appears in the nuclei. See Luger, A. and Lauda, E., Med. Klin., Berlin, 1926, **22**, 415, 456, 493.

**Oxydase,** see **Oxidase.**

**Oxygen Consumption.** A method is described for epidermis separated from dermis by heat (Baumberger, J. P., Suntzeff, V. and Cowdry, E. V., J. Nat. Cancer Inst., 1942, **2**, 413–423.

**Oxyntic Cells** (G. *Oxyntos*, making acid), an unsatisfactory term for the parietal cells of the stomach because it implies actual manufacture of acid.

**Oxyphil** (G. *oxys.*, acid + *philos*, fond) same as acidophilic. The term is commonly applied to the coloid cells of the parathyroid and thyroid which are colored with "acid" dyes such as eosin.

**Ozokerite,** see **Ceresin Imbedding.**

**Pacinian Corpuscles** can best be located by naked eye inspection of the abdominal viscera of a freshly killed cat as small elongated, cigar shaped bodies situated just within the tunica serosa which appear china white because they have a very poor blood supply. Fix in **Zenker's Fluid** and color with **Mallory's Connective Tissue** stain for general purposes or employ **Bodian's** method for nerve fibers.

**Pal-Weigert Method,** see **Weigert-Pal.**

**Palitzsch's Borax-Boric Acid Buffers** (Clark, W. M. The Determination of Hydrogen Ions. Baltimore: Williams and Wilkins, 1928, 717 pp.) Prepare: (1) M/20 borax solution by dissolving 19.0715 gms. $Na_2B_4O_7$ 10 $H_2O$ in 1 liter aq. dest. (2) A solution containing M/5 boric acid and M/20 NaCl by dissolving 12.368 gms. $H_3BO_3$ and 2.925 gms. NaCl in 1 liter aq. dest. To make buffer of the desired pH mix 1 and 2 in the proportions indicated.

| pH | (1) M/20 Borax | (2) M/5 Boric Acid, M/20 NaCl |
|---|---|---|
| 9.24 | 10.0 | 0.0 |
| 9.11 | 9.0 | 1.0 |
| 8.98 | 8.0 | 2.0 |
| 8.84 | 7.0 | 3.0 |
| 8.69 | 6.0 | 4.0 |
| 8.60 | 5.5 | 4.5 |
| 8.51 | 5.0 | 5.0 |
| 8.41 | 4.5 | 5.5 |
| 8.31 | 4.0 | 6.0 |
| 8.20 | 3.5 | 6.5 |
| 8.08 | 3.0 | 7.0 |
| 7.94 | 2.5 | 7.5 |
| 7.88 | 2.3 | 7.7 |
| 7.78 | 2.0 | 8.0 |
| 7.60 | 1.5 | 8.5 |
| 7.36 | 1.0 | 9.0 |
| 7.09 | 0.6 | 9.4 |
| 6.77 | 0.3 | 9.7 |

French, R. W. Stain Techn., 1930, 5 87-90; 1932, 7, 107-108 recommended the use of these buffers for the range pH 9.2-8.2 but he made them up in a different way.

**Palladium.** Histochemical detection based on reaction between palladium and *p*-Dimethylaminobenzyl-idenrhodanin in neutral formalin or alcohol fixed tissues (Okamoto, K., Mikami, G. and Nishida, M., Acta Scholae Med. Univ. Imp. in Kioto, 1939, 22, 382-387).

**Panchrome** is a modification by Pappenheim (Folia haematol., Arch., 1911, **11**, 194) of the Giemsa stain. Add 0.75 gm. of the panchrome powder (Grübler) to 75 cc. pure methyl alcohol and 25 cc. acid free glycerin at 60°C. After filtering keep in glass stoppered bottle. Use after May-Grünwald fixation as described for Giemsa after methyl alcohol fixation. According to Slider and Downey (McClung's Microscopical Technique, p. 329) it gives better coloration of neutrophilic granules and metachromasia of mast granules than the plain Giemsa's stain but "some delicacy is lost, and the cells are more likely to be muddy."

**Pancreas.** This organ lends itself very well to microscopic examination in the fresh state. The classic which everyone seeking technical details should consult is Bensley, R. R., Am. J. Anat., 1911, **12**, 297-388. The techniques for **Blood Vessels** and **Nerve Endings** are those employed generally and are described under these headings. No particular difficulties will be encountered in their adaptation to the pancreas. It may be helpful however to consult Beck, J. S. P. and Berg, B. N., Am. J. Path., 1931, **7**, 31-35 on the blood vessels. The same holds for the **Connective Tissue** components. Epithelial parts of the pancreas can routinely be examined in a preliminary way with the other parts in tissues fixed in **Formalin-Zenker** and stained with **Hematoxylin and Eosin.** For details see **Zymogen, Ducts** and **Islets of Langerhans.**

**Pancreatin** digestion method for spleen (Kyes, P., Am. J. Anat., 1901, **1**, 37-43).

**Paneth Cells.** Influence of fasting on (Klein, S., Am. J. Anat., 1905-1906, **5**, 315-330). To observe storage and discharge phases examine in guinea pigs 24 and 6 hrs. after feeding (Klein, S., Am. J. Anat., 1905-06, **5**, 315-330). By combining DeGalantha's amyloid stain with mucicarmine, Paneth granules are colored green and mucous granules red (Hertzog, A. J., Am. J. Path., 1937, **13**, 351-360).

**Pappenheim,** see **Panchrome, Kardos-Pappenheim, Methyl Green-Pyronin** and **May-Giemsa Stains.**

**Parabenzoquinone,** as a fixative for mitochondria (Baker, J. R., Nature, 1932, **130**, 134; Sircar, S. M., J. Roy. Micr. Soc., 1935, **55**, 238-244).

**Paracarmine** (Mayer). Dissolve 1 gm. carminic acid, 0.5 gm. aluminium chloride and 4 gms. calcium chloride in 100 cc. 70% alcohol. Warm slightly, if required. Allow to settle and filter. Tissues to be stained should not be alkaline or contain much lime (Lee, p. 147).

**Paraffin Imbedding.** For routine it is more convenient than celloidin imbedding. Thinner sections can be cut and it is easier to make them in series. Paraffin imbedding is quicker and the blocks

being dry are easily stored in a smaller space.

After the specimen has been cleared (see **Clearing**) it is placed in paraffin held at a temperature just sufficiently high to keep it melted. For ordinary purposes a paraffin with melting point of 56–58°C. is employed; but 60–62°C. is sometimes selected for very thin sections and 52–54°C. for thick ones. Paraffins of low melting points are described by Waterman, H. C., Stain Techn., 1939, **14**, 55–62. When it is desired to give the imbedding medium more firmness than 60–62°C. paraffin, use is occasionally made of **Rubber Paraffins** *or* **Ceresin.** Under **Clarite** is described a mixture of paraffin and clarite for use in hot weather when thin sections are demanded. Routine paraffin infiltration is best done in wide mouthed glass bottles or jars in an incubator held at the proper temperature. Excessive temperatures harden and shrink the tissues. The paraffin over each specimen should be changed at least once to insure removal of the xylol or other clearing agent. If this removal is incomplete difficulties will be later encountered in crystallization of the paraffin block and in sectioning. The time necessary for infiltration will depend on the size of the tissue and its penetrability. Five to 6 hours is about the average with limits of 2 to 24 hours in special cases. See special treatment for **Teeth** and **Bone.**

For actual imbedding, folded paper containers have now been rather generally replaced by glass dishes. Watch glasses (Syracuse preferred) are satisfactory; but Petri dishes, the inner sides of which are not quite vertical but slope outward slightly from the base, are better. First smear a little glycerin evenly over the bottom and sides of the dish. Then pour in a little paraffin, a thin layer of which will harden so that when the tissue is placed in the dish, it will not come in contact with the bottom. It is customary to orient the tissue so that the surface to be cut first is next to the bottom of the dish. Quickly pour in more paraffin until the tissue is covered to a depth of say 6 mm. Hold the dish in ice water until the surface of the paraffin has hardened just to the point when on immersion in the iced water the surface will hold its shape and not run. However too rapid cooling of paraffins of high melting point may cause cracks in the surface and even in the depth of the blocks. After a few minutes the paraffin block slips out easily because the glycerin prevented it from sticking. When several different specimens are imbedded in the same dish identify each by partly imbedding near it a small strip of paper bearing its number. Finally some of the paraffin is cut away from each tissue so that it can be conveniently filed away but it is important not to remove too much paraffin.

**Paraffin Sections.** 1. *Blocking.* If the specimen is a slice of tissue it was trimmed at the time of fixation into a quadrangular form with each edge and surface parallel to the opposite one. If the specimen is a cross section of a tubular structure the cutting will be more difficult. Heat the metal holder of the microtome, gently press the surface of the paraffin block against it and harden in iced water. The surface of tissue, protected by the most paraffin (which is the upper surface, remote from bottom of the dish, as it was imbedded), should be next to the holder and as far as possible evenly equidistant from the surface of the holder. Unless there is plenty of paraffin between the tissue and the holder, difficulties will be encountered if it becomes necessary to remount the block on subsequent occasions to cut more sections. Since the slice of tissue is of even thickness its outer surface will be evenly parallel to the sweep of the knife so that the tissue included in a given section will be approximately the same distance from the surface of the block and equally subjected to fixation and subsequent technique.

2. *Cutting.* The knife should cut from long side to opposite long side. Trim the edges of the paraffin block so that it will have to pass through an even layer of paraffin at least 5 mm. wide both before and after it enters and leaves the tissue. When more paraffin is cut away it may be later needed if more trimming is required to make the sections into straight ribbons. The sides of the tissue should also be protected by layers of paraffin which are parallel and of even thickness. The object of all this is for the knife to cut through the paraffin and tissue squarely and for it to encounter as nearly as possible equal resistance. The resistance of the paraffin at the sides will, however, always be less than that of the paraffin plus the tissue at the center. For this reason it may be necessary to cut away most of the paraffin from the sides.

But all specimens are not rectangular slices of tissue of uniform thickness. Spherical bodies are easy to cut but the sections obtained are very difficult to flatten. Specimens containing large cavities are troublesome because the

paraffin in the cavities offers so little resistance. In such cases celloidin imbedding is advised. When a part of the tissue is brittle and the rest soft it is best to orient the tissue so that the knife passes through the soft part first. In orientation of fairly large objects a beam of light passed through the paraffin block from an arc lamp or other powerful source is of great assistance. For very minute objects a method described by Fry (H. J., Anat. Rec., 1927, **34**, 245–252) is suggested. For refractory tissues, like yolk laden eggs, McClung (p. 40) suggests hydration. The block is trimmed until the imbedded tissue is exposed when it is soaked in water for several hours. This reduces friability and brittleness and good sections may often be obtained.

Temperature and humidity are factors in securing a good ribbon by making one section stick evenly to the next in series. Sometimes a little boiling water near at hand will help but it should not be necessary if the tissue has been properly infiltrated with paraffin of the right melting point which set firmly when cooled. Static electricity, causing the ribbon to adhere in a troublesome way to surfaces, is partly dependent upon difference in density of tissue and paraffin. But the most important factor in obtaining excellent sections is have the microtome in good working order and the knife sharp (see **Sharpening**). For ordinary purposes sections should be cut 6 microns thick. To mount them on slides first smear carefully cleaned slides (see **Slides**) with **Albumen-Glycerin**, cover with aq. dest. and gently heat over an alcohol lamp if a slide warmer is not available. Then mark the slides with a diamond point pencil and leave for about 6 hrs. in a drying oven at 40–45°C.

**Parafuchsin,** see **Pararosanilin** (Magenta O).

**Paraganglion,** see **Aortic.**

**Paraldehyde** is paracetaldehyde, a polymer of acetaldehyde employed in **Dioxan** fixative and in other ways.

**Paraloidin,** see **Celloidin.**

**Paramagenta,** see **Pararosanilin** (Magenta O).

**Paramylum,** a form of carbohydrate store in lower plants (Taylor in McClung, p. 221).

**Pararosanilin** (Magenta O) (CI, 676)—basic rubin, parafuchsin and paramagenta—This is triamino - triphenyl - methane chloride, the chief component of most **Basic Fuchsins.**

**Parasites.** These range all the way from ultramicroscopic viruses to organisms a yard or more long. Microscopic techniques for viruses are given under **Cytoplasmic Inclusions, Elementary Bodies,** and **Nuclear Inclusions.** Certain Gram negative intracellular insect or arachnid transmitted bacteria - like microorganisms are called **Rickettsia** and require special methods for their demonstration. See also **Bacteria** and **Spirochaetales, Fungi, Piroplasma** and **Protozoa.** A search for such small parasites involves not only an examination of tissues but also of body fluids including **Blood, Feces, Gastric Contents, Urinary Sediment,** etc. When the parasites are scarce resort is made to methods of **Concentration.** Elementary orientation in respect to the larger animal parasites (metazoa) is provided by the following classification (according to Stiles) from Stitt (p. 387) which has been slightly modified.

1. Body more or less dorsiventrally flattened..... 3
   Body in cross section ordinarily round..... 2
2. Body never annulated, without legs or jaws.... 4
   Body annulated (at least possesses mouth parts), breathes usually through tracheal system, adults with jointed legs or other appendages.. 6
3. Intestine present without anus, 1 or 2 suckers, body not segmented. (In liver, lungs, blood, intestine rarely elsewhere—flukes) Trematoda
   Intestine absent, 2 or 4 suckers on head, body of adults segmented, tissue usually contains calcareous bodies, adults (tapeworms) in intestine, larvae (bladder worms) elsewhere Cestoda
   Intestine and anus present, sucker on posterior end, body annulated like earthworm, in upper air passages or externally (leeches, blood suckers).....Hirudinea
4. Intestine absent, armed rostellum present, very rare in human intestine, thorn headed worms Acanthocephala
   Intestine present, but no armed rostellum Nematoda 5
5. Intestine rudimentary in adults, no lateral chords, rare in human intestine (hair snakes or horse hair worms).....Gordiacea
   Intestine present with lateral chords, common in intestine, muscles, lymphatics, etc. (round worms).....Eunematoda
6. Six legs in adult, wings in most species, larvae annulated, breathe by trachea, adults ectoparasites, occasionally under skin, in wounds, intestine or bladder (insects).....Insecta
   Eight legs in adult, 6 in larva, head and abdomen coalesced, ectoparasites, may burrow under skin or live in hair follicles (ticks, mites, etc.).....Acarina
   Four claws about mouth, larvae encysted in various tissues, adults occasionally in nasal passages (tongue worms).....Linguatulidae
   Numerous legs, occasionally in nasal passages and intestine (thousand leggers).....Myriapoda

See **Tapeworm Proglottids, Trematodes, Taenia, Ticks, Insects, Endamœba, Trichinella, Glychrogel.**

**Parenchymatous Degeneration,** see **Cloudy Swelling.**

**Parhemoglobin,** a kind of hemoglobin which crystallizes in same fashion but is insoluble in alcohol (Mallory, p. 135).

**Paris Blue,** see **Spirit Blue.**

**Paris Violet,** see **Methyl Violet.**

**Parlodion,** a derivative of pyroxylin (see **Celloidin**).

**Pectins,** macromolecular properties, test for (Hueper, W. C., Arch. Path., 1942, **33**, 267–290). See **Ruthenium Red.**

**Pentose Nucleotides,** identifiable by ultraviolet absorption spectra maximum about 2600 Å. Their high concentration appears to be correlated with the generally noted basophilia of young tissues (Caspersson, T. and Schultz, J., Nature, 1939, **143**, 602–603).

**Pepsin,** microchemical determination:

1. Freeze gastric mucous membrane of freshly killed pig. Keep at −10°C. Cut cylinders of tissue (2.5 mm. in diameter) with sharp cylindrical borer vertical to surface. Mount cylinders with muscle down on a piece of cardiac mucosa or on stiffened gelatin. Freeze with $CO_2$. Cut sections at 25 microns. Make enzyme determinations on section and correlate with structure in adjacent sections and with known distribution of cell types at different distances from lumen. This shows that chief cells are the source of the pepsin (Holter, H. and Linderstrøm-Lang, K., C. rend. Trav. Lab. Carlsberg, 1935, **20** (11) 1–32).

2. Make extract of tissue, mix with buffers at suitable pH, apply to gelatin surface of Eastman lantern slide plate, incubate, wash gelatin surface, fix in 20% formalin, stain with acid fuchsin or Delafield's hematoxylin and observe sites of proteolytic activity evidenced by clear spots. Test is positive for 0.0001 –0.0002 gm. stomach of young amblystoma weighed wet. Details of this ingenious technique, also applicable with slight modification for trypsin, are given by Dorris (F., J. Exp. Zool., 1935, **70**, 491–527). See also **Peptidase** and **Dipeptidase.**

**Pepsinogen,** antecedent of pepsin in body chief cells of stomach. For staining reaction and discharge by vagal stimulation, *see* Bowie, D. J. and Vineberg, A. M., Quart. J. Exper. Physiol., 1935, **25**, 247–257.

**Peptidase** can be localized in centrifuged marine eggs by direct enzymatic analysis of fragments containing different cytoplasmic components using a procedure essentially the same as that advocated by Linderstrøm-Lang and his associates. It occurs in the hyaline ground substance and is not bound to the granular material (Holter, H., J. Cell. and Comp. Physiol., 1936, **8**, 179–199). Similar studies with amebae indicate, likewise, association with cytoplasmic matrix (Holter, H. and Kopac, M. J., J. Cell. and Comp. Physiol., 1937, **10**, 423–427). These techniques are likely to be of wide usefulness. Peptidase has been localized in gastric and duodenal mucosa of the pig by Linderstrøm-Lang and Holter (K. and H., C. rend Trav. Lab. Carlsberg, 1935, **20** (11), 42–56). See also Mauer et al. (J. Nat. Cancer Inst., 1941, **2**, 278). An excellent critical discussion of the histological distribution of peptidase is provided by Blaschko and Jacobson (Bourne, pp. 207–216).

**Perdrau's Modification.** Bielschowsky's silver method for reticulum as detailed by Bailey, P. and Hiller, G., J. Nerv. & Ment. Dis., 1924, **59**, 337–361. Fix in 10% formalin. Wash in running tap water 12–24 hrs., then in several changes aq. dest., 24 hrs. more. Cut frozen sections, 15–25 μ, and leave in aq. dest. 24 hrs. 0.25% aq. potassium permanganate, 10 min. Wash in aq. dest. Decolorize in equal parts 1% oxalic acid and 1% acid potassium sulphite. Wash in several changes aq. dest. over night. Treat with following solution 40–60 min.: Add 2 drops 40% sodium hydroxide to 5 cc. 20% silver nitrate. Just dissolve ppt. with ammonia. Dilute to 50 cc. with aq. dest. and filter. Wash sections rapidly with aq. dest. Reduce in 20% formalin in tap water, 30 min. Wash in aq. dest. Tone with gold chloride and continue as in **Laidlaw's Method.** Reticulum, black; collagen reddish. This is intended primarily for nervous system, see Bailey and Hiller's, Fig. 3.

**Perényi's Fluid.** 3 parts 95% alcohol, 4 parts 10% aq. nitric acid, 3 parts 0.5% chromic acid is according to Lee (p. 32) an important fixative for embryos, segmenting eggs, etc.

**Perfusion.** The technique of washing through the blood vessels with a fluid is one of wide usefulness. It is in general the same but varies somewhat depending upon what is to be perfused. The apparatus consists of a bottle capable of holding at least 1000 cc. equipped with an outlet near the bottom or a bent glass tube siphon connected by a rubber tube about 6 feet long with a glass **Cannula.** An artery clamp placed about 1 foot from the cannula will serve as a shut off.

If one wishes to perfuse a mouse the

best way is to tie a small cannula into the ventricle, if it is the abdominal organs of a guinea pig the following procedure is advised: Kill the animal with chloroform if this anesthetic will not interfere with the results as is seldom the case. Cut carotids and jugular veins to partly exsanguinate the animal. Clip away sternum and most of the ribs. Displace left lung, expose thoracic aorta and free a portion of it from surrounding tissue. Pass moistened ligature thread behind aorta. Make with scissors a small slit in wall of aorta not at right angles to it but directed into it and downward (toward tail) being careful not to cut more than ½ through it. Insert wet cannula into the slit with slight rotatory motion until the constriction in the cannula is about 1 cm. within the aorta. Then bring the two ends of the thread together and tie the cannula in place. Remove clamp from rubber tube and allow fluid to flow in from bottle suspended about 4 feet above cannula, open right auricle to permit free exit of fluid. It may be necessary to clamp inferior vena cava just above diaphragm and increase pressure somewhat. Sometimes it is helpful to vary pressure by opening and closing clamp. After 4 or 5 minutes open abdomen and examine organ which it is desired to perfuse. The absence of blood color in it and the color of the perfusate (if colored) are indicators of completeness of the operation. The pancreas and the liver will swell considerably but this may not be a disadvantage.

**Pericapillary Cells,** or pericytes, are closely applied to, or wrapped about, the endothelium of blood capillaries. The designation relates to position only and it includes cells of several sorts from much branched Rouget cells to simple fusiform muscle cells and connective tissue cells. Methods of silver impregnation and beautiful illustrations are provided by Zimmermann, K. W., Zeit. f. Anat., 1923, **68**, 29–109. The myofibrils in contractile pericapillary cells can be stained supravitally with janus green, (Bensley, R. R. and Vimtrup, R., Anat. Rec., 1928, **39**, 37–55). Valuable data can be obtained by microdissection of the living tissues (Zweifach, B. W., Am. J. Anat., 1937, **60**, 473–657).

**Pericardium.** Special dissections of bands of fibers in pericardium (Popa, J. T. and Lucinescu, E., J. Anat., **67**, 78–107). Methods for study of absorption of substances placed in pericardial sac (Drinker, C. K. and Field, M. E., J. Exper. Med., 1931, **53**, 143–150).

**Peritoneal Fluid.** Cells present (Webb, R. L., Am. J. Anat., 1931–32, **49**, 283–334; Folia Haemat., 1933, **51**, 445–451).

**Periodontium,** see method for **Teeth** and **Jaws.**

**Peritoneum.** Outlines of mesothelial cells blackened with silver nitrate (Pumala, R. H., Anat. Rec., 1937, **68**, 327–338, good illustrations). Exudate cells stained vitally with lithium carmine (Maximow, A. A., Cowdry's Special Cytology).

**Permeability.** This is a fundamental property for the study of which there are many microscopic techniques. The idea that what goes in and what comes out through the plasma membrane (see **Cell Membranes**) *always* depends upon the character of the particular substance and of the membrane is fallacious. By his method of observing *in vivo* the ruffle **Pseudopodia** of macrophages and cancer cells W. H. Lewis (Am. J. Cancer, 1937, **29**. 666–679) has enabled us to see that materials can be drawn into the cytoplasm in invaginations of the plasma membrane which lose connection with the outside so that when the isolated membranous investments disintegrate the materials are liberated in the cytoplasm without ever traversing the intact surface plasma membrane. This is the converse of observations made possible by the direct examination of secreting acinous cells of the pancreas by W. P. Covell (Anat. Rec., 1928, **40**, 213–223) which show secretory products leaving the cell in protrusions of the plasma membrane. These later become pinched off, the membranes disintegrate and the product is set free in the lumen. See literature review (Blinks, L. R., Ann. Rev. Physiol., 1942, **4**, 1–24).

**Peroxidase.** This enzyme catalyses oxidation of several oxidizable substrates in presence of peroxide. It is most abundant in plants being usually prepared from horse-radish. In mammals it occurs in mammary glands and in milk. In the *peroxidase reaction,* so commonly employed in the study of leucocytes, a colored product is formed in the presence of peroxide from a suitable substrate, benzidine or alpha naphthol. Blaschko and Jacobson (Bourne, p. 197) remind us that it is still uncertain that this reaction in leucocytes demonstrates a true peroxidase because it is relatively stable to heat.

1. Alpha naphthol-pyronin (Graham, G. S., J. Med. Res., 1916, **30**, 231–242). Fix blood smears in 9 parts 95% alcohol and 1 part formalin freshly prepared, 1–2 min. Wash in water and flood with: alpha naphthol (Merck's "recrystallized" or "Reagent"), 1 gm.; 40% alcohol, 100 cc.; hydrogen peroxide,

0.2 cc. for 4–5 min. Wash in dish of running water, 15 min. Stain in: pyronin 0.1 gm.; anilin oil, 4 cc.; 40% alcohol 96 cc., 2 min. Wash in water. Stain in 0.5% aq. methylene blue (Grübler's BX), ½–1 min. Wash in water, blot, mount in neutral balsam. Fresh smears should be used.

2. Benzidine-methylene blue (Graham, G. S., J. Med. Res., 1918, **39**, 15–24). Fix as above. Wash in water. Treat 5–10 min. in 0.2% hydrogen peroxide in 40% alcohol saturated before using with benzidine, 5–10 min. Wash and stain with methylene blue.

3. Benzidine-safranin (Sato, A. and Shoji, K., J. Lab. and Clin. Med., 1927–28, **13**, 1058–1060). Dry blood smear in air. Flood the slides with solution A (0.5% copper sulphate). After 1 minute pour off solution but do not wash or dry slides. Apply solution B (rub up in a mortar 0.2 gms. benzidine with a few drops distilled water. Then add 200 cc. aq. dest. and filter. To filtrate add 4 drops 3% hydrogen peroxide) for 2 min. Then wash in tap water. Stain with solution C (1% safranin in aq. dest), 1 min. Wash in tap water and dry. Peroxidase granules are colored blue in granular leucocytes and the nuclei orange red.

4. Nitroprusside-benzidine (Goodpasture, E. W., J. Lab. & Clin. Med., 1919, **4**, 442–444). To make the stain dissolve 0.05 gm. sodium nitroprusside in 2 cc. aq. dest.; add 100 cc. 95% alcohol; 0.05 cc. benzidine C.P.; 0.05 gm. basic fuchsin and 0.5 cc. hydrogen peroxide. Cover well dried blood smear with known amount of stain, 1 min.; add equal volume aq. dest. plus hydrogen peroxide, 3–4 min.; rinse thoroughly in water and blot dry. Shows many blue granules in granular leucocytes and few in monocytes. Nuclei are colored red. To increase intensity of stain dilute with a little less aq. dest. and stain longer. Method can be used for frozen sections of material fixed in formalin and preserved in 80% alc. A modification of this stain has been proposed by Beacom (J. Lab. & Clin. Med., 1925–26, **11**, 1092–1093) with hydrogen peroxide omitted and basic fuchsin doubled.

5. Benzidine-Giemsa (Armitage, F. L., J. Path., 1939, **49**, 579–580). Fix smears in 96% alcohol containing 10% formol freshly made up. Flood with benzidine mixture (0.75 gm. benzidine in 500 cc. 40% ethyl alcohol. Filter. Add 7 cc. 3% $H_2O_2$, mix by shaking immediately before using) 2 min. for fresh films, longer for older ones. Wash in 40% alcohol until definite yellow granules are seen in granular leucocytes. Absolute alcohol and dry in incubator. Counterstain with dilute Giemsa, wash in water, blot and dry.

6. Benzidine for paraffin sections (McJunkin, F. A., Anat. Rec., 1922–23, **24**, 67–76). After fixation of small pieces in 10% formalin imbed quickly in paraffin; 70% alcohol, 1 hr.; acetone, 30 min.; benzol, 20 min.; paraffin, 20 min. Mount thin sections in usual fashion. Deparaffinize in benzol 20 sec., acetone, 10 sec. Water, few seconds. Drain off water, apply mixture (80% alcohol, 25 cc.; benzidine, 0.1 gm.; hydrogen peroxide, 2 drops) diluted with 1 or 2 parts aq. dest., 5 min. Water, 5 min.; hematoxylin, 2 min.; water, 1 min., 0.1% aq. eosin, 20 sec.; 95% alcohol, 30 sec.; abs. alcohol, 5 sec. Clear in xylol and mount in balsam.

Note: In above methods a blue counterstain tends to obscure the blue peroxidase reaction.

**Peroxydase,** see **Peroxidase.**

**Pętérfi,** see **Double Imbedding,** and **Osmic Acid Method** for nerve fibers.

**Petrunkevitch's Fixatives:** *Cupric-phenol.* Stock solution A = aq. dest., 100 cc.; nitric acid (c.p. sp. gr. 1.41–1.42), 12 cc.; $Cu(NO_3)_2 \cdot 3\ H_2O$, 8 gm. Stock solution B = 80% alcohol, 100 cc.; phenol crystals, c.p. 4 gm.; ether 6 cc. Employ 1 part A with 3 parts B. Fix 12–24 hrs. Wash in 70% alcohol. *Cupric-paranitrophenol.* 60% alcohol, 100 cc.; nitric acid (same), 3 cc.; ether 5 cc.; cupric nitrate (same), 2 gm.; paranitrophenol, c.p. crystals, 5 gm. Time unspecified. Wash in 70% alcohol. Said not to harden tissues like ordinary fixatives. May be followed by all common stains. (Petrunkevitch, A., Science, 1933, **77**, 117–118).

**Petrunkevitch's Fluid** is sat. mercuric chloride in aq. dest., 300 cc., abs. alc., 200 cc.; acetic acid, 90 cc.; and nitric acid, 10 cc.

**pH,** see **Hydrogen Ion Indicators.**

**Phagocytosis.** There are numerous methods for the demonstration of this phenomenon from which to choose.

1. In **Vaginal Smears** (which see), made after intercourse, neutrophilic leucocytes can be observed in the act of engulfing individual spermatozoa. C. R. Stockard, in Cowdry's Special Cytology, 1932, **3**, 1611–1629, has described this remarkable process as seen in the living state. "A leucocyte comes in contact with a spermatozoon which with its tail is longer than the leucocyte. The leucocyte by stretching and contracting finally takes into itself the entire spermatozoon, the tail being wound in a circular fashion within the cell body."

2. In temporary mounts of bacteria and **Leucocytes** (which see) phagocytosis can be followed in detail. Differences in the behavior of neutrophiles from seriously ill and normal persons have been described.

3. Under **Vital Staining** will be found many techniques which permit the observation of the phagocytosis of inanimate particulate materials by macrophages. A graphic demonstration of the immunologic control of phagocytosis of erythrocytes by these cells can be provided by using a method described by Bloom, W., Arch. Path. & Lab. Med., 1927, 3, 608–628.

**Phenol Compounds,** see **Azo Reaction, Indo Reaction.**

**Phenolase,** see **Oxidase.**

**Phenoloxidase,** see criticism of **Dopa Oxidase** reaction.

**Phenolphthalein.** This compound of phthalic acid with phenol and sulfuric acid is an important indicator. Closely related to it is cresolphthalein.

**Phenosafranin** (CI, 840)—safranin B extra—This is the simplest of the safranins. It has been used by Moore, E. J., Science, 1933, 77, 23–24 for staining fungi on culture media or in host tissue.

**Phenyl Methane Dyes.** The hydrogen atoms of methane can be replaced by phenyl groups and it is possible to add amino groups to the benzene rings. See di-phenyl methanes, di-amino tri-phenyl methanes, tri-amino tri-phenyl methanes, and hydroxy tri-phenyl methanes.

**Phenylene Blue,** see **Naphthol Blue R.**

**Phenylene Brown,** see **Bismark Brown Y.**

**Phloroglucin** is 1,3,5-trihydroxybenzene. It is obtained in the form of a yellowish white crystalline powder. It protects the organic components of tissues so that acids can be used in higher concentrations for decalcification. Make sat. aq. sol. phloroglucin and add 5–25% of the acid.

**Phloxine** (CI, 774)—erythrosin BB or B extra, new pink.

**Phloxine B** (CI, 778)—cyanosine, eosin 10B, phloxine TA, N or BB—Conn (p. 154) explains that this differs from phloxine in possessing 4 in place of 2 chlorine atoms in phthalic acid residue of molecule. This phloxine B is the one ordinarily used. See **Eosins.**

**Phloxine Ta, N** or **BB,** see **Phloxine B.**

**Phloxine-Azure.** This resembles Mallory's phloxine-methylene blue. Stain sections after Bouin or Zenker fixation in 2.5% aq. phloxine, 15 min.; wash in water and stain in 0.1% aq. azure A, 30 min.; wash in water, differentiate in 95% alc. plus few drops xylene colophonium; dehydrate in abs., clear in xylol and mount. Particularly good for bone marrow. (Haynes, R., Stain Technology, 1926, 1, 68).

**Phloxine-Methylene Blue.** Mallory (p. 86) recommends that phloxine be employed in place of eosin in the following method because it gives (as Conn suggested) a more brilliant color. Deparaffinize sections of Zenker fixed material in usual way. Remove mercury with 0.5% iodine in 95% alcohol 5–10 min. and the iodine with 0.5% aq. sodium thiosulfate (hypo) 5 min. Wash thoroughly in water. 2.5% aq. phloxine in paraffin over 1 hr. or more. Cool stain, drain and rinse in water. Take 5 cc. 1% methylene blue on 1% borax, 5 cc. 1% aq. azure II, add 90 cc. aq. dest., filter onto the sections. Pour on and off several times. After required time differentiate in 100 cc. 95% alcohol plus 2–5 cc. 10% colophony (rosin) in absolute alcohol. Control differentiation under microscope. Dehydrate in several changes abs. alc. Clear in xylol and mount in balsam. Nuclei and bacteria, blue; collagen, etc. bright rose. The method yields beautiful preparations of intranuclear inclusions in yellow fever and is extensively used for many purposes.

**Phosphatase.** Gomori, G., Proc. Soc. Exp. Biol. & Med., 1939, 42, 23–26. Fix thin slices of tissue in 95% alcohol about 24 hrs. Decalcification is not feasible for acids destroy phosphatase. Imbed in celloidin or in paraffin. In case of the latter exposure to 56–60°C. in paraffin for 2 hrs. is not harmful. Before next step protect paraffin sections after deparaffinization by dipping in 0.5–1% celloidin in alcohol-ether and by hardening celloidin by dipping in 90% alcohol. Incubate sections 2–5 hrs. at 37°C. in following substrate: 1 part 2% aq. sodium glycerophosphate, 1 part 2% aq. calcium nitrate and 6–8 parts aq. dest. Wash and store until next step in dil. sol. calcium nitrate. Visualize ppt. by one of 2 methods. (1) Expose to direct sunlight in 0.5% aq. silver nitrate, 5–30 min., or to light from ultra violet lamp. When desired color is attained rinse in aq. dest. and fix in 1–2% aq. sodium hyposulphite. Sites of phosphatase rich golden brown color. (2) Transfer to 1–2% aq. cobaltous nitrate, 5 min. Rinse in several changes aq. dest. Change black cobalt phosphate to black cobalt sulphide by immersing in very dilute sol. yellow ammonium sulphide (few drops in Coplin jar) few minutes. Wash. After either visualization, sections can be counterstained as desired. If incubation is to be at definite pH, consult original account which also shows good illustrations. It is well to

contrast surface epithelium of small intestine, very rich in phosphatase, with gastric mucosa containing a trace or none at all of it. This is a very valuable, much used method.

Attention is called to the earlier demonstration of phosphatase in bone by Robison (R., Biochem. J., 1923, **17**, 286–293) and to recent discussion by Blaschko and Jacobson (Bourne, pp. 217–221). The distribution of phosphatase in some normal tissues is indicated in colors by Kabat, E. A. and Furth, J., Am. J. Path., 1941, **17**, 303–318. For phosphatase in elementary bodies of vaccinia virus, see Macfarlane, M. G., and Salaman, M. H., Brit. J. Exp. Path., 1938, **19**, 184; Hoagland, C. L. et al., J. Exp. Med., 1942, **76**, 163–173. See **Kidney**.

**Phosphate Solutions.** A method for the direct observation of the effect of buffered phosphate solutions on a thin layer of living, vascular tissue in moat chambers introduced into the rabbit's ear is described by Abell, R. G., Anat. Rec., 1935–36, **64**, 51–73.

**Phosphine** (CI, 793)—leather yellow, xanthin—a basic xanthene dye used as a microchemical test for nucleoproteins by Schumacher, J., Zentralbl. Bakt., Abt. I. Orig., 1922, **88**, 362–366. Phosphine 3 R is fluorchrome for lipids.

**Phospholipid Content** of white blood cells (Boyd, E. M., J. Lab. & Clin. Med., 1935–36, **21**, 957–962).

**Phosphomolybdic Acid Hematoxylin** (Mallory's, see McClung, p. 406). Fix in Zenker's fluid, imbed in paraffin and remove mercury with iodine. Rinse in water. Phosphomolybdic acid hematoxylin at room temperature 12–24 hrs. or at about 54°C. 2–3 hrs. (That is hematoxylin 1 gm., phosphomolybdic acid crystals 2 gm., aq. dest. 100 cc. Requires several weeks to ripen or ripening may be immediate after addition of 5 cc. 1% aq. potassium permanganate.) Wash in water. Decolorize in 95% alc.; dehydrate in abs. Clear in xylol and mount in balsam. Collagenic fibers deep blue. To counterstain first color 5–10 min. in 0.5% aq. acid fuchsin, drain and stain directly in the hematoxylin.

**Phosphorus.** The histochemical detection of phosphorus is a matter of great importance but the techniques are open to much criticism. Lison (pp. 113–120) has reviewed the whole question and advises two techniques as vigorously specific for phosphorus in the ionic form: (1) Angeli (A., Riv. di Biol., 1933, **10**, 702) using plant material treats sections for 20 min. with: ammonium molybdate, 3 gm.; aq. dest., 20 cc.; 30% aq. hydrochloric acid, 20 cc.; reduces in N/50 stannous chloride, rinses quickly in aq. dest., washes longer in 2.5% aq. ammonia which results in elements containing phosphorus being colored blue green. (2) Winter and Smith (L. G., and W., J. Physiol., 1922, **56**, 227–231). See **Radiophosphorus.**

**Phosphotungstic Acid Hematoxylin.** (Mallory's, see McClung, p. 403) Fix in *Zenker's fluid* and remove mercury from sections with iodine or 0.5% sodium hyposulphite. Rinse in water. 0.25% aq. potassium permanganate, 5–10 min. Wash in water. 5% aq. oxalic acid, 10–20 min. Wash carefully in several changes of water. Phosphotungstic acid hematoxylin, 12–24 hrs. (To make this dissolve 0.1 gm. hematoxylin by heat in 50 cc. aq. dest., when cool add 2.0 gm. phosphotungstic acid dissolved in 50 cc. aq. dest. Requires a few weeks to ripen. Ripening can be done at once by addition of 10 cc. 0.25% aq. potassium permanganate). 95% alc., 30 sec.; dehydrate quickly in abs. Clear in xylol and mount in balsam. Fibroglia, myoglia, neuroglia and fibrin, deep blue; ground substance, cartilage and bone, yellowish to brownish red; coarse elastic fibers, purple.

Mullen, J. P. and McCarker, J. C., Am. J. Path., 1941, **17**, 289–291 suggest the following procedure for nervous tissues fixed in *formalin*. Tissues stored in 4% aq. formalin for several years give good results. After fixation in 4%, cut blocks 5 mm. or less in thickness. Wash for 6–12 hrs. in running water. Dehydrate to include 95% alcohol as usual. Complete dehydration in 2 changes *n* butyl alcohol, 4 hrs. each (but absolute alcohol xylol is satisfactory). Imbed in paraffin directly from *n* **Butyl Alcohol** (which see).

Treat sections for 2 hrs. or longer in following mordant: Dissolve 5 gms. chromium chloride (green crystals obtainable from General Chemical Co., New York) in 100 cc. aq. dest. and add 5 cc. glacial acetic acid. This dark green solution soon becomes purple black but is usable after many weeks. Rinse in aq. dest. Stain, as above, with phosphotungstic acid hematoxylin.

**Photodynamic Action** of thiazine dyes on vaccine virus may be due to red or infra red rays (Hirano, N. and Sayama, K., Arch. exp. Med., 1936, **13**, 324–332).

**Photoelectric Microphotometer.** This apparatus has been developed in The Barnard Free Skin and Cancer Hospital by Stowell, R. E., J. Nat. Cancer Inst., 1942, **3**, 111–121 to measure the light absorbed as a result of the specific coloration of tissue components. It consists of a lamp, microscope, photocell and

equipment for amplification and recording. The particular component investigated has been **Thymonucleic Acid** as demonstrated in the epidermal cells of mice by the Feulgen reaction. The method is one of wide usefulness and will probably be employed as a means of securing quantitative data from many microchemical reactions.

**Photoxylin,** see **Celloidin.**

**Phrenosin** is a **Cerebroside.**

**Phthalein Indicators.** Table giving relative reactions of the several organs and tissues after vital staining (Rous, P., J. Exper. Med., 1925, **41**, 739–759). See **Indicators** of pH.

**Physiological** solutions. These are intended for the examination of living cells with a minimum of change. Blood serum, or plasma, is an unnatural medium for any living cells except those naturally intravascular as shown by the fact that alone and undiluted it is a poor medium for tissue culture. *Physiological saline* is for mammals 0.85–0.9% aqueous NaCl and for amphibians about 0.65% aqueous NaCl. For others see **Ringer, Ringer - Locke, Locke - Lewis** and **Tyrode. Normal solutions** (which see) are different.

**Pia Mater.** Perivascular nerves. Wash out blood by vascular perfusion with saline solution or by rinsing nonperfused tissue with saline. Fix with 10.5% citric acid in 20% formalin preferably by injection. Dissect out blood vessels of pia under binocular microscope. Wash in aq. dest. twice and place in 20% aq. silver nitrate 2 hrs. Pass through 4 changes 20% formalin in Petri dishes each containing about 100 cc. Transfer directly to ammoniated silver nitrate made by adding conc. ammonia (28%) drop by drop to 20% aq. silver nitrate using 3 drops more than amount required to dissolve ppt. Observed under the microscope the nerves "come up" slowly and when they are dark enough transfer to 20% ammonia water for 1–2 min. Wash in aq. dest. plus few drops glacial acetic acid. Tone in 0.2% aq. yellow gold chloride 30–60 min. Wash in water, dehydrate in 3 changes 95% alcohol, clear in carbol-creosote-xylol mixture and mount in balsam (Penfield, W., Am. J. Path., 1935, **11**, 1007–1010).

**Pianese Method.** Much used a generation ago for study of cancerous tissue. Pianese, G., Beitr. z. Path. Anat., u. Allg. Path., 1896, Suppl. I, 193 pp.

**Picric Acid** is a very important ingredient of several fixatives. It penetrates rapidly and serves to some extent as a mordant like potassium bichromate. *See* **Bouin's fluid.**

**Picro-Carmine** (Ranvier). There are many sorts most of them based on Ranvier's original formula: Add carmine (dissolved in ammonia) to sat. aq. picric acid to saturation. Evaporate to ⅓ original volume, cool, filter out ppt. and evaporate filtrate to dryness. The resultant red crystalline powder is picro-carmine. Make a 1% aq. sol. for staining. If overstained decolorize with 0.2% hydrochloric acid. This is an excellent and very popular stain. It colors keratohyalin very brightly (Lee, p. 146).

**Picro-Formol,** see **Bouin's Fluid.**

**Picro-Indigo-Carmine** is a much used stain. Lee (p. 433) advises 3 parts sat. indigo-carmine in 70% alc. and 1 part sat. picric acid also in 70% alc.

**Picro-Nigrosine** for muscle. After alcohol or Bouin fixation, stain sections in sat. nigrosine in sat. aq. picric acid. Muscle yellow, connective tissue black.

**Picro-Sulphuric** fixative, see **Kleinenberg's.**

**Pigments,** general reviews: Bergmann, E. Ergeb. d. Physiol., 1933, **35**, 158–300; Lederer, E., Biol. Rev., 1940, **15**, 273–306 (invertebrates). See **Bacterial, Bile Pigment, Bilirubin, Biliverdin, Carotin, Carotinalbumins, Carotinoids, Chromolipoids, Cytochrome, Hematin, Hematoidin, Hematoporphyrin, Hemofuscin, Hemoglobin, Hemosiderin, Iron Pigments, Lipochrome, Lipofuscin** (wear and tear pigment), **Malarial, Melanins, Parhemoglobin, Porphyrins, Rhodopsin, Sulfmethemoglobin.**

**Pinacyanol** (CI, 808)—sensitol red—A basic xanthene dye of the cyanine group. Proescher, F., Proc. Soc. Exp. Biol. & Med., employed the Eastman Kodak Co. product of which a 0.1–0.5% solution in absolute ethyl or methyl alcohol for 5–10 sec. stains frozen sections brightly. Wash in water, mount in glycerine. Chromatin, blue violet; protoplasm, purple; connective tissue, red; elastic tissue, black violet; muscle, bluish violet to purple; amyloid, red; etc. Hetherington, D. C., Stain Techn., 1936, **11**, 153–154, used pinacyanol as a supravital stain for mitochondria in blood cells.

**Pinocytosis,** a term introduced by Lewis (W. H., Bull. Johns Hopkins Hosp., 1931, **49**, 17–26) to indicate drinking by cells as opposed to phagocytosis, or eating by cells. It means (Lewis, W. H., Am. J. Cancer, 1937, **29**, 666–679) microscopically visible drinking, not submicroscopic "sipping" which Meltzer termed "potocytosis". By this process in tissue cultures proteins and other substances that do not diffuse into the cells are engulfed by wavy ruffle pseudopodia. The cell membrane, which first invests the globules of fluid taken into the cytoplasm, later disappears and the

fluid becomes part of the cytoplasm. Can be best seen in cultures of cancer cells of which an excellent moving picture is available for distribution by the Wistar Institute of Anatomy at Philadelphia.

**Piroplasma** (L. pirum, pea + G. plasma, a formed thing). Piroplasmas are pear shaped parasites of red blood cells causing diseases of great importance in domestic and other animals but not as yet found in man. They can be colored by any good blood stain. **Giemsa** and **May-Giemsa** are recommended.

**Pituitary.** The microscopic techniques for this conductor of the endocrine symphony are obviously too numerous to mention. Consult each issue of the Quart. Cum. Index Med.

To differentiate 2 classes of acidophiles in the cat a modification of Heidenhain's "azan" modification of Mallory's connective tissue stain is proposed by Dawson, A. B. and Friedgood, H. B., Stain Tech., 1938, **13**, 17–21. T. Maxwell, Jr. (*ibid*, 93–96) proposes a modification especially designed for the basophiles and Koneff, H. H. (*ibid*, 49–52) one for the rat. In addition all within the space of a few months, Lewis, M. R., and Miller, C. H., Stain Techn., 1938, **13**, 111–114 give following directions to demonstrate 2 types of granular cells in the *pars nervosa*. Fix in 3% aq. potassium bichromate 2 parts and half sat. corrosive sublimate in 95% alc. 1 part, 12–24 hrs. with 1 change. Dehydrate to 70% alcohol to which add few drops iodine. Change each day until solution retains color. Dioxan, 8–24 hrs., 3 changes. Dioxan + little paraffin. Paraffin 4 changes. Cut sections 4 microns and deparaffinize. Stain 0.25% aq. acid fuchsin 30 min. Then 1–24 hrs. in Mallory's stain (aq. dest., 100 cc.; anilin blue, 0.5 gm.; orange G, 2 gm. and phosphotungstic acid, 1 gm.). Differentiate in 95% alcohol until no more color comes out. Abs. alc., xylol, balsam. To identify microglia in neurohypophysis see Vazquez-Lopez, E., J. Anat., 1942, **76**, 178–186.

**Plants.** Except for pathogenic **Bacteria** and **Fungi**, technique for plants does not come within the scope of this book. However much is to be learned, especially in microchemistry, from many methods employed by botanists and the reader is advised to consult Johansen, D. A. Plant Microtechnique. New York: McGraw-Hill, 1940, 523 pp.

**Plasma Cells.** Since plasma cells (of the Marschalko type) are mainly identified by recognition of a small area near the nucleus which does not stain as intensely as the rest of the cytoplasm with basic dyes, it is important to use a technique which reveals basophilia. In practice Giemsa's stain, or a good coloration with hematoxylin and eosin, is generally sufficient. Unna used the term "plasma cell" for almost any kind of cell with much plasma including macrophages so that the designation Unna's plasma cell is almost meaningless.

**Plasma Membrane**, see **Cell Membranes.**

**Plasmalogen.** A component of the cytoplasm which gives a positive Feulgen test (Bourne, p. 22).

**Plasmosin**, the gel and fiber forming constituent of the hepatic cell. Method of isolation and properties (Bensley, R. R., Anat. Rec., 1938, **72**, 351–369).

**Plasmosome.** The true nucleolus staining with "plasma" or "acid" dyes, that is to say, red with eosin in the hematoxylin and eosin combination. The plasmosome apparently does not make any direct contribution to chromosome formation. Acidophilic nucleoli are quite different from certain cytoplasmic granules which Arnold called "plasmosomes" and mitochondria termed "plastosomes" by Meves.

**Plastics,** chemistry and physics of (Bartoe, W. F., J. Tech. Meth., 1940, **20**, 6–11). In museum work (Kramer, F. M., J. Tech. Meth., 1940, **20**, 14–23). As substitute for cover glasses (Suntzeff, V. and Smith, I., Science, 1941, **93**, 158–159; Russell, W. O., J. Tech. Meth., 1942, **22**, 65–70). As mounting media (Hutner, S. H., Stain Techn., 1941, **16**, 177). As substitute for quartz for light conduction (Williams, R. G., Anat. Rec., 1941, **79**, 263–270).

**Platelet Counts.** *Total* counts can be made in plasma. Walker and Sweeney (T. F. and P. A., J. Lab. & Clin. Med., 1939, **25**, 103–104) proceed as follows: Moisten inside white blood cell pipette by drawing in and expelling 1.1% aq. sodium oxalate. Immediately draw in fresh blood to 0.5 mark, then oxalate solution to mark 11. Shake vigorously. Place heavy rubber band around pipette to close ends. Centrifuge pipette ½ min. at 1,600 revolutions, or the shortest time to draw red blood cells into its stem. Stand pipette vertically to permit red blood cells to settle into stem (about 2 hrs.). Gently expell red blood cells by blowing and count platelets in clear supernatant solution.

Another method (Buckman, T. E. and Hallisey, J. E., J.A.M.A., 1921, **76**, 427–429) is to prick the finger, or ear, through a drop of 0.1% brilliant cresyl blue in physiological saline. The fluid plus blood is mounted and the number per red cell is counted. If there is one platelet per 20 reds and there

are 6 million reds per c. mm., then there are approximately 300,000 platelets per c. mm. of blood which is a normal count. The number may exceed 1 million in myelogenous leukemia.

A choice can be made from many platelet staining solutions: (1) Buckman, T. E., and Hallisey, J. E., J.A.M.A., 1921, **76**, 427: Glucose, 6.0 gm.; sodium citrate, 0.4 gm.; aq. dest., 100 cc. Filter, add 0.02 gm. toluene red (dimethyldiamidotoluphenazin) then 0.1 gm. crystal violet. Heat gently to 60°C. 5 min.; cool and centrifuge at 2000 revolutions per min. for 10 min. Filter supernant fluid twice through 2 thicknesses filter paper. Preserve solution by adding 0.2 cc. formaldehyde. (2) Kristenson, A., Acta Med. Scandinav., 1922, **57**, 301: Urea, 10 gm.; sodium citrate, 2.5 gm.; corrosive sublimate, 0.005 gm.; brilliant cresyl blue, 0.5 gm.; and aq. dest., 500 cc. (3) Ottenberg, R. and Rosenthal, N., J.A.M.A., 1917, **69**, 999: 3% aq. sodium citrate to which 1:500 cresyl blue or 1:500 methyl violet is added and filtered before staining. (4) Pratt, J. H., J.A.M.A., 1905, **45**, 1999: Sodium citrate, 3.8 gm.; aq. dest., 100 cc.; formaldehyde, 0.2 cc.; brilliant cresyl blue 0.1 gm. (5) Wright, J. H. and Kinnicutt, R., J.A.M.A., 1911, **56**, 1457: A. Brilliant cresyl blue, 1 gm.; aq. dest., 300 cc. B. Potassium cyanide, 1 gm.; aq. dest., 1400 cc. Keep A in ice box. For use 2 parts of A and 3 parts of B are mixed and filtered.

A *differential* platelet count, in which 4 classes are recognized, is described by Olef (I., Arch. Int. Med., 1936, **57**, 1163).

**Platelets.** These can best be seen in the dark field in mounts of fresh blood and of fresh blood first treated with **Anticoagulants.** The contrast between the two is instructive. It is important to remember that when held under observation in preparations sealed with vaseline for a considerable time, platelets may become elongated and exhibit a superficial resemblance to parasites. Data on the rate of disintegration of platelets are provided by Olef, I., J. Lab. & Clin. Med., 1936–37, **22**, 128–146. In blood regeneration atypical platelets may be encountered measuring as much as 25–50 $\mu$ in length (Tocantins, M., Medicine, 1938, **17**, 175–258). The contained granules are easily stained supravitally and in smears. Excellent coloration of platelets in sections are given by Wrights and Kingsley's methods (see **Megakaryocytes**).

**Platino-Acetic-Osmic** mixture, see **Hermann's Fluid.**

**Platinum.** Intravenous injections of colloidal solutions of platinum in rabbits are described by Duhamel, B. G., C. rend. Soc. de Biol., 1919, **82**, 724–726.

**Platinum Chloride** is the name usually given to hydro-chloroplatinic acid. It is used occasionally as an ingredient of fixatives.

**Platyhelminthes** is the phylum of flatworms. The two classes of important parasites are the **Cestodes** and **Trematodes.** See **Parasites.**

**Pleuropneumonia.** Staining of organisms. Stain paraffin sections 4 microns thick of tissue fixed in Zenker, Bouin, absolute alcohol or Carnoy's fluid brought down to water directly in *Mallory's phosphotungstic acid hematoxylin* (18–24 hrs.) without preliminary treatment with permanganate and oxalic acid. Do not wash but blot nearly dry and dehydrate rapidly in absolute alcohol, clear in xylol and mount in balsam. Organisms in lungs appear as deep blue masses of mycelial threads (Turner, H. W., Austral. J. Exp. Biol. & Med. Sci., 1935, **13**, 149–155).

**Plimmer's Bodies,** see **Bird's Eye Inclusions.**

**Polarization Optical Methods.** Employing strong polarized light it is possible to obtain data on the orientation of molecules in membranes, see **Nerve Fibers, Muscle Fibers,** etc.

**Polarized Light** is said to be better than Marchi and Sudan III methods for study of myelin degeneration of peripheral nerves (Prickett, C. O. and Stevens, C., Am. J. Path., 1939, **15**, 241–250). Used in study of mitochondria and Golgi apparatus (Monné, L., Protoplasma, 1939, **32**, 184–192).

**Polarizing Microscope.** Any ordinary microscope can be adjusted for crude polarization studies by use of a polarizer and analyser. *See* **Polarization Optical Methods.**

**Polaroid.** This is a polarizing material made up of extremely minute crystals of quinine sulphate periodide. A nitrocellulose film containing the crystals all oriented in the same direction can be mounted between sheets of glass with a total thickness fo about 3 mm. See Bourne, p. 26.

**Poly-Azo Dyes.** Chlorazol black E, sudan black B.

**Polychromatic Erythroblasts,** see **Erythrocytes,** developmental series.

**Polychrome Methylene Blue.** Literally many colored, but actually in this case two colored. It is a methylene blue which contains, in addition to the blue itself, large amounts of azures especially A and B. These are redder than methylene blue and are partly responsible for

the metachromatic staining (G. *meta*, beyond + *chroma*, color) given by polychrome methylene blue. The color is beyond and different from the simple blue by reason of its marked reddish tint. It is usually better to purchase the polychrome methylene blue rather than to make it. If it has to be made dissolve 1 gm. methylene blue in 100 cc. 0.5% aq. $NaHCO_3$; place in steam sterilizer 1½ hrs.; cool and filter (McClung, p. 334). It should be a good methylene blue. Goodpasture's (E. W., J.A.M.A., 1917, **69**, 998) recipe for polychrome methylene blue is: Boil 400 cc. aq. dest. + 1 gm. methylene blue and 1 gm. potassium carbonate for 30 min. Cool and add 3 cc. acetic acid and shake dissolving ppt. Boil gently down to 200 cc. volume (5 min.). Cool. Eosinates spectra and staining potency (Lillie, R. D. and Rce, M. A., Stain Techn., 1942, **17**, 57–63). See also Lillie, R. D., Stain Techn., 1942, **17**, 97–110 for acid oxidation methods of polychroming.

**Polyvinyl Alcohol,** macromolecular properties (Heuper, W. C., Arch. Path., 1942, **33**, 271).

**Ponceau B,** see **Biebrich Scarlet,** water soluble.

**Ponceau R, RG, G, 4R, 2RE, NR, J, FR, GR,** see **Ponceau 2R.**

**Ponceau 2R** (CI, 79).—Brilliant ponceau G, lake ponceau, new ponceau 4R, ponceau R, RG, G, 4R, 2RE, NR, J, FR, GR, scarlet R, xylidine ponceau 3RS.—An acid mono-azo dye which may be the ponceau de xylidine called for in **Masson's Trichrome Stain.**

**Ponder's Stain** for **Diphtheria Bacilli,** which see.

**Pontamine Sky Blue 5BX,** see **Niagara Blue 4B.**

**Poppy Seed Oil,** reactions in tissue to fat stains after various fixations (Black, C. E., J. Lab. & Clin. Med., 1937–38, **23**, 1027–1036).

**Porphyrins.** There is no specific histochemical reaction. Identification depends on fluorescence and spectra. In tissues the fluorescence in solution is red or orange in ultraviolet light but pure porphyrins in solid condition are never fluorescent. Even in solution in the tissues they may not be fluorescent. Harderian glands of mice with low susceptibility to cancer give intense red fluorescence (Figge, F. H. J., Strong, L. C. and L. C. Jr. and Shanbrom, A., Cancer Research, 1942, **2**, 335–342). Graffin, A. L., Am. J. Anat., 1942, **71**, 43–64 gives technique for rat with valuable illustrations. These glands are the best organs in which to study ooporphyrin. The histological distribution of porphyrins in humans is fairly well known, especially in zones of calcification in newborns. Literature is reviewed by Dobriner and Rhoads (K. and C. P., Physiol. Rev., 1940, **20**, 416–468). For identification of uroporphyrin and coproporphyrin see Lison (p. 256). See **Hematoporphyrin.**

**Postmortem Change.** These are alterations in structure due to autolytic and osmotic changes. The rate of autolysis is very rapid in some organs such as the pancreas which are enzyme producers. It is relatively slow in the walls of elastic arteries in which the proportion of inanimate components (elastic and collagenic fibers) is high. In the case of tissues which cannot be immediately fixed certain precautions should be taken to minimize postmortem change. See **Agonal Changes, Artifacts, Fixation,** and **Small Intestine, Necrosis, Necrobiosis.**

**Potocytosis,** a term introduced by Meltzer to designate submicroscopic "sipping" of fluid by cells. See **Pinocytosis.**

**Pottenger's Dilution Flotation** method, see **Concentration** of bacteria.

**Potassium,** Histochemical methods.

1. Policard, A. and Pillet, D., Bull. d'Hist. Appl., 1926, **3**, 230–235, have suggested that potassium and sodium probably occur as chlorides and that their conversion to sulphates by treating the sections with sulphuric anhydride fumes makes them more stable and better able to withstand the high temperature of **Microincineration** which see.

2. Marza, V. D., Bull. d'Hist. Appl., 1935, **13**, 62–71 has modified Macallum's well known technique. Fix small pieces of tissue in 96% pure alc. in the ice box. Make paraffin sections. To eliminate the possibility of the presence of iron leave control sections 5 min. in freshly prepared sol. yellow ammonium sulphate. Wash in aq. dest., dehydrate, clear and mount in neutral balsam. There should be no ppt. Make up following solutions: A. Cobalt nitrate, 5 gm.; aq. dest., 10 cc.; glacial acetic acid, 2.5 cc. B. Sodium nitrite, 25 gm.; aq. dest., 36 gm. To A add 41 cc. of B and use immediately. If delay is necessary keep in ice box and filter before using. Cover test sections with this for 2 hrs. in a closed Petri dish to avoid evaporation. Wash slowly in 50% alc. to remove every trace of reagent. Plunge in ammonium sulphate solution 3 min. Wash in aq. dest. to remove ammonium sulphate. Dehydrate, clear and mount. Examine illustrated paper by Marza and Chiosa (V. D. and L. T., Bull. d'Hist. Appl., 1935, **13**, 153–177)

on application of this method to the problem of ovogenesis.

3. Gersh, I., Anat. Rec., 1938, **70**, 311–329 has also modified Macallum's method. It involves the making of similar paraffin sections as for **Chloride**, which see. Transfer these to a fairly large cool room (−1° to +1°C.) and remove paraffin and petroleum ether as for chloride. Cover with 12% sodium cobalti-nitrite solution of Bülman (Treadwell, F. P., Analytical Chemistry, vol. 1, 4th English Ed. translated by W. T. Hall, New York, John Wiley & Sons, Inc., 1916, p. 81). Decant fluid, mount in glycerin in same way and examine. Crystals of sodium potassium cobalti-nitrite are just visible with oil immersion lens. They are short yellow rods with rounded ends in a diffuse pale yellow background soluble at room temperature.

4. Carer-Ccmes, O., Zeit. f. wis. Mikr., 1938, **55**, 1–6 has advised histochemical demonstration of potassium by Siena orange (K. Hollborn), which is sodium paradipicrylamine. Deparaffinize sections of neutral formalin fixed tissue. Place in Siena orange solution, as received ready for use from Kollborn, 2 min. Then 10% HCl 3 min. Wash twice in aq. dest. 10 min. Blot with filter paper and dry at 37°C. Mount in thickened cedar oil. Tissues containing potassium, orange; others, pale yellow or unstained.

5. Radioactive potassium can be easily measured in tissues and cells. There is 40% penetration of red blood cells *in vivo* (Mullins, L. J., Noonan, W. O. and T. R. and Halge, L., Am. J. Physiol., (1941, **135**, 93–101). See **Radiopotassium.**

**Pressure.** Increase in pressure beyond a certain limit, somewhat characteristic for particular cells (300–1000 atmospheres), brings about a liquefaction of the plasmagel which can be directly observed microscopically or determined by certain measurements like action potential for nerve fibers. Danielli (Bourne, p. 38) has expressed the opinion that the factor causing inhibition of movement may, in all cases, be increased hydration of protein molecules and that the method of increased pressure may be of great value to large scale and micro-biologists.

**Price-Carr Reaction,** see **Carr-Price Reaction.**

**Primula R Water Soluble,** see **Hofmann's Violet.**

**Primulin** (CI, 812)—primuline yellow—An acid thiazole dye used in fluorescence microscopy (Pick, J., Zeit. Wis. Mikr., 1935, **51**, 338–351).

**Primuline Yellow,** see **Primulin.**

**Prolactan.** Methods for assay (Bates, R. W., Cold Spring Harbor Symposium on Quantitative Biol., 1937, **5**, 191–197).

**Promyelocytes,** see **Leucocytes,** developmental series.

**Prontosil** as a vital dye (Carter, W., Science, 1939, **90**, 394).

**Propylcarbinol,** see **n-Butyl Alcohol.**

**Prostate.** This organ cannot be examined microscopically in vivo and supravital staining has not proved very fruitful. The cutting and staining of sections is the conventional method. It is important that the blocks of tissue fixed be oriented with great care, and that microscopic and gross observations be correlated. For normal size and weight see Moore, R. A., Am. J. Path., 1936, **12**, 599–624 and for age changes a chapter by the same author in Cowdry's Problems of Ageing, Baltimore: Williams & Wilkins, 1942, 936 pp. Since the structure of the prostate exhibits so many local differences there is a danger of erroneous conclusions from incomplete examination. In their classic paper on the rat-prostate cytology as testis hormone indicator Moore, C. R., Price, D. and Gallagher, T. F., Am. J. Anat., 1930, **45**, 71–107 secured best results after fixation in **Bouin's Fluid** and staining with **Harris' Hematoxylin** and **Eosin.**

Swyer, G. I. M., Cancer Research, 1942, **2**, 372–375 has checked with satisfactory results the Schultz test for cholesterol by chemical analyses. He has also outlined a method for measuring the color in the Liebermann-Burchardt reaction. For singly refractile fat in the epithelial cells see Gylling, P., Acta Path. et Microb. Scan., 1941, **18**, 247–258.

To demonstrate the ducts (Le Duc, I. E., J. Urol., 1939, **42**, 1217–1241) in autopsy material lay open prostate by incising length of anterior commissure and express secretion from ducts by gentle massage and careful sponging. Locate orifices of ducts with aid of a dissecting microscope. Inject celloidin solution into them through No. 26 or 27 gauge hypodermic needle fitted with tapering solder tip. Then macerate with hydrochloric acid and remove all except casts of the ducts. See his illustrations.

A method for demonstrating arterial supply is described and illustrated in some detail by Flocks, R. H., J. Urol., **37**, 524–548. Inject internal iliac arteries of a fresh cadaver with equal parts barium sulphate and water at 200–250 mm. mercury pressure. But beforehand cut small branch of superior

vesical artery to relieve pressure in prostatic vessels. Remove prostate with sufficient surrounding tissue. Cut gland into 5–6 sections each about 1 cm. thick. Dehydrate in ascending alcohols and clear in oil of wintergreen (methyl salicylate).

Examination of corpora amylacea by various methods is described by Moore, R. A., Arch. Path., 1936, 22, 24–40.

**Protargol.** This is a light brown protein silver compound containing approximately 8% silver. To demonstrate phagocytosis by the reticulo-endothelial system fine suspensions may be injected intravenously (Askanazy, M., Aschoff Path. Anat., Jena, 1923, 1, 183) but the method is not recommended by Foot (McClung, p. 115). Protargol is also used for staining of paraffin sections (Bank, E. W. and Davenport, H. A., Stain Techn., 1940, 15, 9–14). See **Silver Methods, Bodian Method.**

**Protease.** A proteolytic leucocytic enzyme which can be demonstrated by a special method in very small amounts of blood (Cooke, J. V., 1932, 49, 836–845). A micromethod for protease is described by Pickford and Dorris (G. E. and F., Science, 1934, 80, 317–319).

**Protein,** see following reactions: **Alloxan, Axenfeld, Azo, Indo, Ninhydrin, Nitro, Nitroprusside, Nitrosamino, Romieu, Xanthroproteic.**

**Proteinase,** determinations (Mayer, M. E., Mider, G. B., Johnson, J. M. and Thompson, J. W., J. Nat. Cancer Inst., 1941, 2, 278).

**Prothrombin,** rapid micro test (Abramson, D. J. and Weinstein, J. J., Am. J. Clin. Path. Technical Suppl., 1942, 6, 1–7):

1. Make M/40 calcium chloride by dissolving 1.11 gms. anhydrous calcium chloride C.P. in 400 cc. aq. dest.

2. Make thromboplastin suspension from brain freshly killed rabbit as described by Quick, A. J. Am. J. Clin. Path., 1940, 10, 222. Dehydrate macerated brain in acetone, dry completely, mix with normal saline (0.3 gm. to 5 cc.) and incubate at 50°C. 15 min. The supernatant turbid fluid is thromboplastin. It must be kept in ice box when not in use.

3. Measure separately in microhemopipettes 10 cc. of calcium chloride sol., of thromboplastin and of blood.

4. After adding blood, mix thoroughly with fine glass rod, tilt gently from side to side until gelation begins, then time end point by passing rod through mass.

*Prothrombin* time (Sherber, D. A., J. Lab. & Clin. Med., 1940, 26, 1058–1061).

**Protoporphyrin** in Harderian glands, see **Porphyrins.**

**Protozoa,** staining in bulk. (Stone, W. S., J. Lab. & Clin. Med., 1935–36, 21, 839–842): Suggested for mucous surface protozoa of man and used at Army Medical School. Thoroughly emulsify 20 cc. feces in 200 cc. 37°C. physiological saline solution. Allow to stand for 5 min. and pour supernatant fluid into two 50 cc. centrifuge tubes. Centrifuge at 1,850 r.p.m. 5 min. Decant supernatant fluids. Examine residue from one, fresh, and to other add 25 cc. **Schaudinn's Fixative.** Mix and leave 24 hrs. Protozoa in cultures and other fluids are to be concentrated by centrifugation and fixed in the same way. Between each of following steps centrifuge organisms and discard supernatant fluid before adding the next. Wash twice in aq. dest. Wash with 70% alcohol plus sufficient Gram's iodine to make it light brown color, 10 min. Wash 70% alcohol 10 min. Stain **Harris' Hematoxylin** 1–24 hrs. Wash tap water. Destain by adding 20 cc. acid alcohol (1% HCl in 70%) controlled by microscope. When desired definition is reached add sufficient ammonia water (6 drops $NH_4OH$ to 50 cc. aq. dest.) to neutralize acid and give bright blue solution. Wash in tap water. Dehydrate 10 mins. in each of 5 alcohols: 70, 95, 95, abs., and abs. Clear in xylol. Mount in balsam. See author's figures.

Levine W. D., Stain Techn., 1939, 14, 29–30 suggests following method to make **Methylene Blue** stains permanent: Wash methylene blue stained smears of protozoa repeatedly in aq. dest. 15 min. to 1 hr. Place in tertiary butyl alcohol 1–2 min. then in 3 or more changes 15 min. each. Pass through xylol to balsam or mount directly in balsam. Other dyes like toluidin blue O, nile blue sulfate, eosin Y, ponceau 2R can likewise be retained.

The protargol method of **Bodian** has been adjusted to protozoa by Cole, R. M. and Day, M. F., J. Parasitology, 1940, 26 Suppl. 29. See also **Parasites, Endamoeba Leishmania, Leucocytozoa, Malaria.** Wenyon, C. M., Protozoology. New York: William Wood, 1926, 1563 pp. is a convenient book of reference. It gives a fine list of blood protozoa. No investigator can afford to ignore the discussion by Wenrich, D. H., J. Parasitol., 1941, 27, 1–28 of alterations in the form of protozoa resulting from variations in microtechnique.

**Prussian Blue** (CI, 1288) is ferric ferrocyanide, a colored salt. It is also known

in commerce as Berlin blue, Chinese blue, Paris blue, Milori blue and Steel blue. An aqueous solution of Prussian blue is a good medium for the injection of blood vessels. It contrasts nicely with carmine. The particles of both are sufficiently large to be held within the endothelium. Deposition of Prussian blue is useful in the localization of drainage of **Cerebrospinal Fluid** (Weed, L. H., J. Med. Res., 1914, **26**, 21–117) and in the microchemical demonstration of **Iron** (Gomori, G., Am. J. Path., 1936, **12**, 655–663). See **Berlin Blue.**

**Pulp of Teeth.** This can be studied *in situ* in undecalcified teeth or in paraffin or celloidin sections of decalcified ones. See **Teeth.** If it is to be examined by itself after removal from the teeth and fixation, attempt to preserve its natural elongated shape. Almost all methods available for other soft tissues are applicable. Wellings, A. W., Practical Microscopy of Teeth and Associated Parts. London: John Bale, Sons & Curnow, Ltd. 1938, 281 pp. gives many of them. See **Teeth, Innervation.**

**Purines.** Silver methods for histochemical detection are according to Lison (p. 185) absolutely useless.

**Psittacosis,** method for staining elementary bodies (Hornus, G. J. P., Ann. Inst. Pasteur, 1940, **64**, 97–116). See other kinds of **Elementary Bodies.**

**Purkinje Cells** of heart. Distend entire heart by injecting fixative through 4 cannulae, in aorta, in pulmonary artery, in superior vena cava, in one pulmonary vein and ligating other vessels. Fix in Zenker's or Bouin's fluid. Sino-auricular node is at junction of superior vena cava and right auricle. Cut blocks perpendicular to the node. Color paraffin sections with Masson's trichrome stain or with hematoxylin and eosin for transitions between Purkinje and cardiac muscle cells. The sharpest differential stain for the former is Best's carmine stain for glycogen (Taussig, H. B., J. Tech. Methods, 1934, **13**, 85–87).

**Purkinje Fibers.** In excising the specimen the presence of Purkinje fibers is localized by the dimpling in a cross section because in the fresh state the Purkinje fibers contract more than the cardiac fibers (Todd, T. W. in Cowdry's Special Cytology, 1932, **2**, 1179). Todd recommends for general purposes Bouin's fixative and Mallory's stain. Safranin light green is good for the intercalated discs (Jordan, H. E., and Banks, J. B., Am. J. Anat., 1917, **22**, 285–338). Techniques for bringing out the Purkinje system particularly of mammalian ventricles are described by Abramson, D. I. and Margolin, S., J. Anat., 1935–36, **70**, 250–259.

**Purpurin** (CI, 1037)—alizarin No. 6, alizarin purpurin—An acid anthraquinon dye. The bright red color of madder-stained bones is due to purpurin carboxylic acid (Richter, D., Biochem. J., 1937, **31**, 591–595).

**Pycnosis** (G. *pyknos*, dense) When the substance of a cell, as seen in stained sections is unusually dense it is sometimes said to be pycnotic. The increase in density is usually accompanied by a decrease in size of cytoplasm and/or nucleus and the nucleus may be hyperchromatic, that is have an increased affinity for stains like hematoxylin and methylene blue. Sometimes pycnotic cells occur singly surrounded by others not in the same condition but they may be present in group. Those in the central nervous system have been called chromophile cells (Cowdry, E. V., Contrib. to Embry., Carnegie Inst., 1917, **11**, 29–41). Information is needed on the cause or causes of pycnosis and on the fate of cells in this condition.

**Pyoktanin Yellow,** see **Auramin.**

**Pyoktaninum Aureum,** see **Auramin.**

**Pyoktaninum Coeruleum,** see **Methyl Violet.**

**Pyronin.** There are 2 pyronins: B (CI, 741) and Y (CI, 439) also known as G. Conn (p. 140) describes them as closely related to diphenyl methanes since they have one carbon atom attached to 2 benzene rings and exhibit similar tendency to quinone structure. Their formula also resembles that of oxazins except that nitrogen of central ring is replaced by CH radical. Pyronin B is tetra-ethyl diamino xanthene and Y is the tetra-methyl compound. Conn (McClung p. 599) advises Y with methyl green in Pappenheim's stain, for the granules of mast cells and the gonococcus in smears of pus. B is satisfactory for most purposes. Only recently has the distinction been made so that most formulae call simply for pyronin. American pyronins are now more concentrated than those imported before 1914. Conn says that allowance should be made for this difference in the proportions of pyronin and methyl green.

Pyronin G is the best supravital stain for the duct system of the pancreas (Bensley, R. R., 1911, **12**, 297–388). It is applied by **Perfusion** a solution of 1:1000 in 0.85% aq. NaCl being used until the pancreas takes a light rose color. Small pieces are then mounted in salt solution and examined. The ducts from the main ones to the centro-acinous cells are sharply stained in red

against an almost colorless background. The ducts may be similarly stained by methylene blue in a concentration of 1:10,000. To obtain a beautiful contrast coloration Bensley injects with a salt solution containing 1:100 pyronin and 1:15,000 janus green. This stains the ducts red and the islets bluish green. The combination of 1:1000 pyronin and 1:15,000 neutral red also demonstrates ducts and islets but without an equally distinct color contrast. The pyronin method for ducts is one of the most useful techniques both for investigation and for class room demonstration.

**Pyrosin B,** see **Erythrosin,** bluish.

**Pyroxylin** (collodion cotton, colloxylin, soluble gun cotton, xyloidin, cellodion wood). It is chiefly cellulose tetranitrite. Mainly used in manufacture of **Collodions, Celloidin, Paraloidin, Photoxylin,** etc.

**Pyrrol Compounds,** see **Nitro Reaction, Nitrosamino Reaction.**

**Quartz Rod Technique** for illuminating living organs. Light can be conducted through fused quartz rods placed on the other side of organs in the living animal in such a way that alterations during functional activity can be directly observed. This method has already been of great value in the study of cyclical activity of the venous sinuses of the spleen. It can be employed in the analyses of many other vital activities. For detailed description see Knisely, M. H., in McClung's Microscopical Technique, New York: Paul B. Hoeber, Inc. 1937, 698 pp. Methyl methacrylate polymer is a plastic suggested as a cheap substitute for quartz (Cole, E. C., Science, 1938, 87, 396–398). Lucite (ethyl methacrylate polymer) is used by Williams, R. G., Anat. Rec., 1941, 79, 263–270.

**Quinoline Dyes.** Only pinacyanol is of apparent value to histologists.

**Quinone-Imine Dyes.** Possess 2 chromophores: indamin-N= and quinoid benzene ring. They are divisible into **Azins, Indamins, Indophenols, Oxazins, Thiazins.**

**Quinone Oximes,** see **Nitroso Dyes.**

**Rabbit Ears,** see **Sandison's Technique** for inserting transparent chambers in.

**Rabl's Fluid** is sat. aq. mercuric chloride, 1 part; sat aq. picric acid, 1 part; aq. dest., 2 parts.

**Radiation.** Methods and results of radiation of normal tissues reviewed (Warren, S. and Dunlap, C. E., Arch. Path., 1942, **34,** 562–608 and earlier papers).

**Radioactive Isotopes** as tracer substances (from Dr. W. L. Simpson of The Barnard Free Skin and Cancer Hospital).

In the 20 years that have elapsed since Hevesy first used a radioactive isotope of lead to trace the lead metabolism of plants, advances in nuclear physics have made available to biologists materials that appear to open up new approaches to a variety of problems limited only by the ingenuity of the investigator and the availability of the tracer substances he desires. Discovery of the phenomenon of artificial radioactivity in 1934 by I. Curie and F. Joliot and the development of the cyclotron by E. O. Lawrence and his associates at the University of California are acknowledged generally to be the chief factors that have produced these important advances.

The assumption is made that an isotope is accepted by tissues without discrimination, and that its distribution, metabolism, and elimination will be the same as that of the non-radioactive form of the element. This appears valid except perhaps for the lightest elements in which relatively great differences of atomic weight exist between the radioactive and the stable isotopes. Although radiations from large (therapeutic) doses of some isotopes do exert profound effects on tissues, the concentration of those employed as tracer substances is usually so low (often less than one part to several billion of the stable isotope) that no tissue changes can reasonably be attributed to the radiation accompanying their decay.

Less than 5% of the cyclotron produced radioactive isotopes have been employed in biological studies. Among the limitations to their use are the following: (1) They are sometimes difficult to obtain. Isotopes that decay rapidly are available only to experimenters near the cyclotron. (2) The rate of decay of unstable isotopes must be slow enough to permit the measurement of their radiation at the end of an experiment. While larger quantities can be employed to offset rapid decay, a limit is soon reached beyond which further increases in concentration is either not possible because of difficulties in preparing them or is not desirable because of the effects produced by radiation of tissues. The length of experiment should not be longer than 5 or 6 times the half life of the element used. (3) The form in which the radioelement is desired places a limit on some investigations. Since they are usually prepared from pure elements or simple compounds, the use of elements in complex forms is limited by the amount of

synthesis that can be accomplished. In some cases synthesis of complex organic compounds can be carried out most readily by the introduction of simple radioactive salts into an animal or plant and the subsequent recovery from the organism of complex substances that contain the radioelements incorporated in their structure.

Three methods of detection of the radioactive isotopes are commonly used:

1. *In vitro method:* Most common is the measurement of the radiation from the isotope with either a Geiger-Müller counter or an electroscope. Tissues to be examined are either ashed and measured or extracted and measured in solution. The Geiger-Müller counter is extremely sensitive but only gross tissue localization is possible, since relatively large amounts of tissue must be extracted.

2. *In vivo method:* Detection and localization of some isotopes that emit penetrating Gamma rays are feasible within the living body by placing a shielded Geiger-Müller counter against the body so that it will receive rays from restricted areas. Thus Hamilton has studied the accumulation of radioiodine in the thyroid gland.

3. *Autoradiography (or radioautography):* Known since 1924 (Lacassagne, A. and Lattès, J. S., C. rend. soc. d. biol., 1924, **90**, 352–353; C. rend. d. l'Acad. d. sc., 1924, **178**, 488–490) this technique secures on photographic emulsions images representing the location of radioactive elements in tissue and organ slices that have been held in contact with photographic films. Photographic records of sections of fixed tissues containing radioelements can be made by simply laying the mounted unstained sections on a photographic plate and leaving them until adequate exposures are obtained. Subsequently sections are stained for comparison with the silver deposit on the developed plate. See distribution of thorium B (a lead isotope) in animal tissues by B. Behrens and A. Baumann (Zeits. f. d. ges. exper. med., 1933, **92**, 241–250). Interesting studies have been carried out also by J. G. Hamilton on the localization of radioiodine in normal and enlarged thyroid glands. The deposition of radiophosphorus and radiostrontium in bones and osteogenic tumors has been autoradiographed by Treadwell, Low-Beer, Friedell and Lawrence. Radiophosphorus distribution in leaves and fruit of plants has been studied by Arnon, D. J., Stout, P. R., and Sipos, F., Am. J. Botany, 1940, **27**, 791. According to Hamilton (J. G., Radiology, 1942, **39**, 541–572), Lindsey and Craig have proved that the method is valuable in the study of phosphorus distribution in insect larvae. Gorbman, A. and Evans, H. L., Proc. Soc. Exper. Biol. & Med., 1941, **47**, 103 have similarly determined the time in embryonic development when the thyroid first accumulates iodine.

Space does not allow further review of the many problems that can be investigated using radioactive tracer substances. See Hevesy, G., Ann. Rev. Biochem., 1940, **9**, 641–662 and Hamilton, J. G., J. Appl. Physics, 1941, **12**, 440–460 and Radiology, 1942, **39**, 541–572. Theoretical considerations are discussed by Hevesy, G. and Paneth, F. A., "A Manual of Radiology," 2nd edition, London: Oxford Univ. Press., 1938, and in a popular review of the development of the cyclotron by Abersold, Paul C., Radiology, 1942, **39**, 513–540. For literature on nearly 400 radioactive isotopes see Seaborg, G. T., Chem. Rec., 1940, **27**, 199–285. The effects of radioelements on growth of cells, tissues, and organisms have been considered by Haven, F. L., and Hodge, H. C., Growth, 1941, **5**, 257–266. The Annual Reviews of Biochemistry, volumes 8 to 11 contain many data on the use of tracer substances. See review of mineral metabolism by Greenberg, D. M., Ann. Rev. Biochem., 1939, **8**, 269–300.

**Radioarsenic** ($As^{76}$) half life 26.8 hrs. Used as a tracer for the distribution of sodium dihydrogen arsenate in rabbit tissues by duPont, O., Ariel, I. and Warren, S. L., Am. J. Syph., Gonor. and Ven. Dis., 1942, **26**, 96–118. Highest concentrations appear in liver, kidney, and lungs. In lower concentration it is found in muscle, bone, and skin. Browne-Pearce tumor tissue takes significant amounts but loses them within 4 days. Elimination is chiefly by kidneys.

**Radiobromine** ($Br^{82}$) half life 34 hrs. Perlman, I., Morton, M. E. and Chaikoff, I. L., Am. J. Physiol., 1941, **134**, 107–113 followed the uptake of very small doses of radiobromine by various tissues of rat and guinea pig. Highest concentrations appear in thyroids in both normal animals and in animals with thyroids made hyperplastic by the pituitary thyrotropic hormone.

**Radiocalcium** ($Ca^{44}$) half life 180 days. Stored almost entirely in bone. Only small traces are found in other tissues (Campbell, W. W. and Greenberg, D. M., Proc. Nat. Acad. Sci., 1940, **26**, 176–180 and Pecher, C., Proc. Soc. Exper. Biol. Med., 1941, **46**, 86–91). Pecher also predicted that strontium is

handled in the body in the same fashion as shown in his experiments. Long half life makes this element rather difficult to work with.

**Radiocarbon** ($C^{11}$) half life 21 min. Short life makes use difficult in many investigations. In spite of this handicap, S. Ruben, M. D. Kamen and their coworkers have used radiocarbon to study $CO_2$ metabolism and photosynthesis in a wide variety of lower animals and plants. Their findings on the nature of photosynthesis, at variance with the long accepted view, afford a nice illustration of the manner in which well planned experiments with the radioisotopes can support or dispel classical assumptions. They showed, for instance, that chlorophyll containing plants can assimilate radiocarbon dioxide in the absence of light and convert it to a carboxylic acid radical attached to a particle of high (approximately 1000) molecular weight. The process is limited in the absence of light, but in the presence of light assimilation continues with a photosynthetic reduction of the carboxylic acid radical to an alcohol group with the liberation of oxygen. This newly formed alcohol radical accepts $CO_2$ in another non-photosynthetic reaction. Successive alternate photosynthetic and non-photosynthetic reductions lead to the building of longer carbon chain radicals on the large enzyme molecule. Presumably these chains eventually split off as simple sugars, etc. See Ruben, S., Hassid, W. Z. and Kamen, M. D. (J. Am. Chem. Soc., 1939, **61**, 661; 1940, **62**, 3443), Ruben, S., Kamen, M. D., Perry, L. H., *ibid*, 1940, **62**, 3450; Ruben, S. and Kamen, M. D., *ibid*, p. 3451. Kamen, M. D. and Ruben, S., J. Appl. Physics, 1941, **12**, 310A suggest the possibility of *in vivo* synthesis of sugars, acetic acid, etc. from radiocarbon as a means of obtaining radioactive substances that are too complex to be synthesized in the laboratory in the time available during the useful life of radiocarbon.

**Radiocarbon** ($C^{14}$) half life estimated to be over 1000 years. Very small quantities are produced but specific activity is high. It may be useful for study of some biological problems.

**Radiochlorine** ($Cl^{38}$) half life 37 min. Used chiefly to investigate rate of chloride ion penetration into various tissues (Manery, J. F. and Haege, L. F., Am. J. Physiol., 1941, **134**, 83–93). The permeability of human erythrocytes to radiochloride ion has been determined and compared with permeability to other non-radioactive ions by Smith, P. K., Eisenmann, A. J. and Winkler, A. W., J. Biol. Chem., 1941, **141**, 555–561. A complete exchange between radiochloride ions of serum and erythrocytes was found within less than 10 min.

**Radiocobalt** ($Co^{57}$) half life—270 days. Little work has been done with this isotope. Copp, D. H. and Greenberg, D. M., Proc. Nat. Acad. Sci., 1941, **27**, 153–157 report on the distribution of minute doses. The bulk of ingested radiocobalt is rapidly excreted. Less than 5% is retained after 4 days. This fraction is found chiefly in pancreas, kidney, spleen, and liver. The interesting question is raised of a possible relation between cobalt retention in the pancreas and the association of cobalt with insulin.

**Radiocopper** ($Cu^{64}$) half life 13 hrs. Distribution in blood serum and red cells has been briefly reported by Yoshikawa, H., Hahn, P. F. and Bale, W. F., Proc. Soc. Exper. Biol. Med., 1942, **49**, 285–289, and J. Exper. Med., 1942, **75**, 489–494. A peak concentration is reached in plasma 2 to 5 hrs. after ingestion when it falls off rapidly. The concentration in red cells continues to increase over 2 days.

**Radioelement 85** (ekaiodine, $85^{211}$) half life 7½ hrs. This element, which does not occur naturally in any known form, has been used by Hamilton and Soley (J. G. and M. H., Proc. Nat. Acad. Sci., 1940, **26**, 483–489) as a heavy homologue of iodine in their studies on thyroid. General behavior resembles iodine in thyroid.

**Radiofluorine** ($F^{18}$) half life 112 min. Volker, J. F., Sagnnaec, R. F. and Bibby, B. G., Am. J. Physiol., 1941, **132**, 707–712 studied distribution in rats and cats after intravenous and intraperitoneal injection of radiofluorine salts. Blood concentration falls rapidly as the concentration in calcium containing tissues rises. Ultimate concentration in calcified tissues is in proportion to their vascularity.

**Radioiodine** ($I^{131}$) half life 8 days. A comparison of the rate of uptake of radioiodine, radiosodium, radiopotassium, radiochloride, and radiobromide has been made in normal human subjects by Hamilton, J. G., Am. J. Physiol., 1938, **124**, 667–678. The rate of gamma ray emission from the hand is followed after the subjects receive the salts by mouth. This indicates the rate at which the radiosalt enters the circulation. Peak absorption is reached within 1½–2 hrs. for all except radiopotassium. Its absorption continues to rise slowly for 4½ hrs.

The metabolism of iodine by the thyroid gland in various physiological

and pathological conditions has naturally attracted much attention. Hertz, S., Roberts, A. and Evans, R. D., Proc. Soc. Exper. Biol. Med., 1938, **38**, 510–513 first demonstrated the rapid uptake of radioiodine in the thyroid gland. Increase of the radiosalt is even more rapid in hyperplastic thyroids. Numerous studies on thyroids of humans have been reported by Hamilton, J. G. and Soley, M. H., Am. J. Physiol., 1939, **127**, 557–572 and *ibid*, 1940, **131**, 135–143. Much of this work was done on the living glands by measuring the gamma radiation from the neck region over the thyroids by a Geiger-Müller counter. These same workers, with the cooperation of Eichorn, K. B., Univ. Calif. Publ. Pharmacology, 1940, **1**, 339–367 demonstrated, by means of the technique of autoradiography, that radioiodine is deposited in the colloid of normal and hyperplastic thyroids. The storage is markedly lower in the colloid of nontoxic goiter, and almost no radioiodine enters cancerous thyroid tissue. Mention has already been made of the work of Gorbman and Evans on determination by autoradiography of time of first storage of radioiodine in the thyroids of embryonic frogs.

**Radioiron** ($Fe^{59}$) half life 47 days. Yield very low. ($Fe^{55}$) with a half life of 4 yrs. has not been much used as yet in biological problems. What follows relates to $Fe^{59}$.

Whipple and his associates have availed themselves of radioiron to show that iron metabolism is controlled by the rate of absorption of the iron salts from the intestine and not by the rate of elimination of the iron already in the tissues. A higher proportion of absorbed iron goes into the formation of hemoglobin in anemic animals than in normal ones. The rate of absorption from the intestine seems to be controlled by the iron content of the tissues, especially of the intestinal mucosa, and not by degree of anemia *per se*. See numerous papers by Whipple, G. H., Bale, W. F., Lawrence, E. O., Hahn, P. F. *et al.*, chiefly in the J. Exper. Med., 1938–1941, much of which is confirmed by Austoni, M. E. and Greenberg, D. M., J. Biol. Chem., 1940, **134**, 27–41 who also demonstrate that the muscles serve as an important storehouse for iron in anemia.

Use of erythrocytes "tagged" with radioactive iron opens up a new approach to many baffling problems in hematology. One recent investigation employing such labeled red cells is that of Chapin, M. A. and Ross, J. F., Am. J. Physiol., 1942, **137**, 447–455 who checked the values for true red cell volume using "tagged" erythrocytes in comparison with results from dye dilution, protein dilution, and the hematocrit. The technique of measuring the activity of such red cells is described by Ross, J. F. and Chapin, M. A., Rev. Sci. Instr., 1942, **13**, 77–80. Erythrocytes can also be labeled with radiophosphorous.

**Radiomanganese** ($Mn^{54}$) half life 310 days. Not much use has been made of this isotope to date but Greenberg, D. M. and Campbell, W. W., Proc. Nat. Acad. Sci., 1940, **26**, 448–452 have observed that 90% of ingested radiomanganese is eliminated in the feces within 75 hrs. The highest retention is in the liver, bones, and muscles.

**Radionitrogen** ($N^{13}$) half life 9.93 min. Short useful life limits study to experimental procedures that are completed within approximately 1 hr. Ruben, S., Hassid, W. Z. and Kamen, M. D., Science, 1940, **91**, 578 have found that it enters into the complex compounds within barley plants which live in air containing it. Whether this represents nitrogen fixation by a non-leguminous plant, or a simple exchange between the radionitrogen and ordinary nitrogen within the plant, is not established as yet.

**Radiophosphorus** ($P^{32}$) half life 14.3 days. Employed more extensively than any other isotope, radiophosphorus is rather easy to prepare and its useful life is long enough to permit most experimental procedures, and short enough to allow ready detection with the Geiger-Müller counter or photographic plate of radiation from its decay. Since no gamma rays are given off during its decay radiophosphorus can not be detected in the intact organism, except in the skin. Also encouraging its use is the fact that phosphorus plays such an extensive rôle in the compounds found in living organisms. In deciding whether to employ radiophosphorus it is helpful to bear in mind the kinds of work in which it has already proved useful.

Chiewitz, O. and Hevesy, G. (Nature, 1935, **136**, 754) were the first to use artificially produced radioactive isotopes in biological research. Initial studies were devoted to investigation of metabolism of $P^{32}$ in rats. Absorption and excretion of $P^{32}$ in experimental animals and humans have been studied by Hevesy and coworkers, Lawrence and associates, and Greenberg and Cohn. At least 70% of ingested $P^{32}$ (as inorganic phosphates) is absorbed from intestine when fed to a fasting subject. The balance is excreted in feces. Glucose and

neutral fat enhance absorption. Intravenous disodium phosphate containing tracer quantities of $P^{32}$ in the phosphate ion: 4–23% is eliminated in 24 hrs. in urine and feces. Rate falls to less than 1% per day after 3d day. The retention of radiophosphorus varies in different tissues. In decreasing order the activity of the element appeared in bone, muscle, liver, stomach and small intestine, blood, kidneys, heart, lungs and brain. The turnover of radiophosphorus in brain is much slower than in other tissues. On basis of weight retention it is highest in bone, liver, intestinal tract, heart, kidneys, lungs, blood, muscle, skin, and brain (in decreasing order). Scott, K. G. and Cook, S. F., Proc. Nat. Acad. Sci., 1937, **23**, 265–272 found that large doses of $P^{32}$ cause decrease in polymorphonuclear leukocytes in circulating blood of chicks, presumably due to selective beta ray irradiation of the bone marrow in consequence of higher absorption and retention in bone. Lawrence and his group (Lawrence, J. H. and Scott, K. G., Proc. Soc. Exp. Biol. & Med., 1939, **40**, 694–696; and Lawrence, J. H., Tuttle, L. W., Scott, K. G., Conner, C. L., J. Clin. Invest., 1940, **19**, 267–271), as result of this finding, compared phosphorus metabolism of normal and leukemic mice. Although the total phosphorus content of lymph nodes, spleen, and liver was about the same in normal and leukemic animals, the proportion of $P^{32}$ in the leukemic animal was distinctly higher, indicating a higher rate of phosphorus metabolism in these animals. Lawrence, J. H., Radiology, 1940, **35**, 51–60 reported the use of radiophosphorus on a group of patients suffering from leukemia and polycythemia. Hevesy, G. and Lundsgaard, E., Nature, 1927, **140**, 275–276 and Arton, C., Sarzana, G., Perrier, C., Santangelo, M. and Segré, E., Nature, 1937, **139**, 836–837 have studied conversion of inorganic phosphates to phospholipids. They observed different rates of synthesis and storage in various organs. Studies on phospholipids using $P^{32}$ as tracer are reviewed by Sinclair, R. G., Biol. Symposium., 1941, **5**, 82–98. Chaikoff and his colleagues (numerous papers in J. Biol. Chem., 1937 and following years) confirmed these results on different animals and extended studies to isolated tissue slices *in vitro*. Jones, H. B., Chaikoff, I. L. and Lawrence, J. H. (J. Biol. Chem., 1939, **128**, 631–634) found different types of malignant tumors had characteristic patterns of phospholipid metabolism not related to cell types. Marshak separated cell nuclei from cytoplasm and observed malignant cell nuclei accumulated more $P^{32}$ than normal nuclei and that relative to cytoplasm malignant nuclei took up more than normal cells, comparing lymphoma cells with normal liver cells (Marshak, A., Science, 1940, **92**, 460–461 and J. Gen. Physiol., 1941, **25**, 275–291). This combination of the techniques using tracers and methods of separating components of cells offers great promise for further investigation. Numerous reports on plant tissues and on insects are also available. Manly, M. L. and Bale, W. F. (J. Biol. Chem., 1939, **129**, 125–134) have described $P^{32}$ distribution in rat bones and teeth. Sognnaes, R. F. and Volker, J. F., Am. J. Physiol., 1941, **133**, 112–120 have studied distribution of $P^{32}$ in parts of the teeth of cats, dogs, and monkeys. Most $P^{32}$ is found in dentin, and little in enamel. Of that in enamel, highest concentration is in outermost layer, suggesting that some minerals reach the teeth by diffusion from saliva. Radiophosphorus may be used to "label" erythrocytes in much the same manner as radioiron is used. Recently Brown, Jr., F. A., Hempelman, Jr., L. H. and Elman, R. have used such "tagged" erythrocytes to determine true blood volume (Science, 1942, **96**, 323–324).

**Radiopotassium** ($K^{42}$) half life 12.4 hrs. Rate of absorption of radiopotassium from the gut was investigated in conjunction with the study of radioiodine by Hamilton. The distribution of injected radiopotassium in tissues of the rat has been studied by Noonan, T. R., Fenn, W. O. and Haege, L. (Am. J. Physiol., 1941, **132**, 474–488). An early concentration of the ion occurs in liver, heart, kidney, lung, diaphragm, and gastrointestinal tract. After equilibrium is reached, most of the radioactivity is present in tissues normally high in potassium, i. e. muscle, skin and viscera. Recently Lyman, C. P., Am. J. Physiol., 1942, **137**, 393–395 employing this isotope has demonstrated an increased permeability of denervated skeletal muscle to potassium ion.

**Radiorhubidium** ($Rb^{86}$) half life 18 days. It is possible that radiorhubidium can be used in place of radiopotassium which is difficult to prepare but no work appears to have been reported as yet.

**Radioselenium** ($Se^{75}$) half life 48 days. McConnell, K. P., J. Biol. Chem., 1941, **141**, 427–437 has reported on the retention of radioselenium in various tissues. 19% is found in the liver; considerable amounts in muscle, intestine, and blood; less in the testis. None is present in

the skin, fur, teeth, and long bones. The element is chiefly excreted by the kidneys.

**Radiosodium** ($Na^{24}$) half life 14.8 hrs. Easiest element to prepare. The yield is very high. Another isotope, $Na^{22}$ has a half life of 3 yrs., and may eventually prove quite useful. To date little use has been made of this longer lived isotope in biology. All references below are to the $Na^{24}$ isotope.

This has been extensively employed by Flexner, Gellhorn, and Pohl to determine rates of placental transfer in mammals. See: Flexner, L. B. and Pohl, H. A., J. Cell. and Comp. Physiol., 1941, **18**, 49–60; Am. J. Physiol., 1941, **134**, 344–349; Gellhorn, A., Flexner, L. B. and Pohl, H. A., J. Cell. and Comp. Physiol., 1941, **18**, 385–392; and Flexner, L. B. and Gellhorn, A., Am. J. Obst. and Gynec., 1942, **43**, 965–974.

**Radiostrontium** ($Sr^{89}$) half life 55 days. Distribution of this isotope in the body is much like radiocalcium. The more intense radiation from this element than from radiocalcium makes it a possible choice for localized radiation therapy of bones and osteogenic tissues. Autoradiographic and other evidence for accumulation in a human osteogenic sarcoma is presented by Treadwell, A. deG., Low-Beer, B. V. A., Friedell, H. L. and Lawrence, J. H., Am. J. Med. Sci., 1942, **204**, 521–530.

**Radiosulphur** ($S^{35}$) half life 88 days. The metabolism of radiosulphur in inorganic salts and in synthetically prepared thiamine chloride (vitamin $B_1$) in normal and vitamin deficient human subjects has been studied by Borsook and his coworkers (Borsook, H., Hatcher, J. B. and Yost, D. M., J. Appl. Phys., 1941, **12**, 325A and earlier papers).

**Ranson Pyridine** method for unmyelinated nerve fibers (Ranson, S. W., Rev. Neurol. & Psychiat., 1914, **12**, 467–474). Fix in absolute alcohol + 1% ammonia, 48 hrs. Rinse in aq. dest. and treat with pyridine, 24 hrs. Wash repeatedly in aq. dest., 24 hrs. 2% aq. silver nitrate at 35°C. in dark, 3 days. Rinse in aq. dest. Reduce in: pyrogallol, 4 gm.; 5% formalin in aq. dest., 100 cc. Wash and imbed in paraffin. This much used technique gives a fine blackening of unmyelinated fibers. See also Ranson, S. W. and Billingsley, P. R., J. Comp. Neurol., 1918, **29**, 313–358; Johnson, S. E., *ibid*, 1928, **38**, 299–314). The latter believes the essential features of the technique to be vascular perfusion with physiological saline solution followed by 1% ammonia in absolute alcohol.

**Ranvier's Gold Chloride** method for nerve endings in muscle, see **Graven's** and **Carey's** methods. See also **Ammonia Carmine** and **Picrocarmine** of Ranvier.

**Reconstruction.** Stereoscopic x-ray method (Morton, W. R. M., J. Anat., 1940–41, **75**, 265–266); wax plate method as applied to the stapes (Anson, B. J., Karabin, J. E. and Martin, J., Arch. Otolaryng., 1939, **29**, 939–973).

**Red B,** see **Oil Red O.**

**Red Corallin,** (CI, 726). Look up in Colour Index.

**Red Violet,** see **Hofmann's Violet.**

**Redox** dyes are those employed in reduction-oxidation potential determinations, see **Oxidation-Reduction Potential.**

**Refractive Index.** Microscopical determination by standard liquids. See paper by Kunz, A. H. and Spulnik, J., Reviewed in J. Roy. Micr. Soc., 1937, **57**, 55.

**Regaud's Fluid.** 3% aq. potassium bichromate, 20 cc.; formalin, 5 cc. When this is used for mitochondria fix tissue for 4 days changing every day and then mordant in 3% aq. potassium bichromate for 7 days changing every second day. It is a fluid that can be profitably employed for many other purposes. Of these see **Giemsa's Stain, Lead, Masson's Trichrome, Romieu Reaction** and **Starch Grains.**

**Regaud's Method** of iron hematoxylin for mitochondria. Fix tissues in Regaud's fluid, mordant, imbed and section as described under **Anilin Fuchsin Methyl Green Method.** Run mounted sections down to water and mordant for 24 hrs. in 5% aq. iron alum. Rinse quickly in aq. dest. (not tap water) and transfer to hematoxylin (made by dissolving 1 gm. hematoxylin crystals in 10 cc. abs. alc. adding 10 cc. glycerin, 80 cc. aq. dest. and allowing to ripen 3 weeks). If traces of iron alum are carried to the stain they will do no harm, but if too much enters the hematoxylin a dense black precipitate will form and ruin the hematoxylin. On the other hand, if the sections are washed excessively in aq. dest. too much of the alum will be removed and the hematoxylin will not stain as intensely as it should. The happy mean must be determined. The hematoxylin should be used over again about 10 times. Differentiate in 5% aq. iron alum under low magnification. Wash in running tap water (not aq. dest.) 1 hr. This should bring out the blue-black color of the hematoxylin stain. Dehydrate, clear and mount. Various counterstains can be used if desired. Consult Meves' beautiful figures of collagenic fibers stained with fuchsin (Meves, F., Arch. f. Mikr. Anat., 1910, **75**, 149–208). This is the

most permanent stain for mitochondria but lacks the color contrast afforded by anilin fuchsin methyl green.

**Reissner's Fiber,** staining reactions of (Jordan, H., Am. J. Anat., 1925, **34,** 427–443).

**Replacement of Tissue** to take the place of that worn out or lost can now be measured more accurately. Though some signs of youth and age of cells can be detected (Chapter 24 in Cowdry, E. V., Problems of Ageing. Baltimore: Williams & Wilkins, 1942, 936 pp.), it is not so easy to determine the percentage actually dying as the percentage of new cells produced to replace them by counting mitoses. Using whole mounts of separated human **Epidermis** from foreskins removed by circumcision Cooper, Z. K., and Schiff, A., Proc. Soc. Exp. Biol. & Med., 1938, **39,** 323–324 have discovered that the production of new cells is rhythmic being greatest at night and least by day. To obtain material, as they did every hour of the day and night, of other human tissues seems impossible. If one wishes to investigate rate of cellular replacement in internal less accessible tissues that are replaceable, take advantage of the fact that the drug, colchicine, permits cells to enter mitosis but arrests the process usually in the metaphase. In consequence of this experimental summation many more mitoses can be counted in a given specimen than would be found if cell division had been completed as usual (See **Mitosis** for the necessary controls). There are no special means for the study of replacement of **Fibers** but careful use of available techniques will probably yield data as to whether the fibers are newly formed or old and practically useless.

Physico-chemical methods are however promising when backed by histological researches. Thus the new bone formed, during the time that **Madder,** or better **Alizarin Red S,** is made available in the circulation can be measured. In adult animals, assuming that the amount of bone remains approximately constant, it can be concluded that the breakdown is at the same rate and in this round about way arrive at a figure for replacement.

Some fats can be conveniently colored with fat soluble dyes which they retain on ingestion and after incorporation in the fatty depots of the body. It should, therefore, be possible to keep animals at a fairly constant weight on a diet containing a certain amount of fat, to substitute for this fat stained fat of the same sort without increasing their weight and to estimate the ratio of stained to unstained fat after a definite interval of time—in other words the replacement. Other possibilities are to employ for the test a fat of melting point quite different from the native body fat of the animals; and fatty acids tagged with radioactive isotopes, see **Fatty Acids.**

The radioactive isotopes, particularly those of **Phosphorus** and **Iron** give somewhat similar clues. The amount of radiophosphorus, for example, accumulating in any particular tissue can be accurately determined. If the supposition is justified that the total amount of phosphorus (radioactive and non-radioactive) remains about the same, then non-radioactive phosphorus must be lost at the rate that the radiophosphorus enters. It is too soon however to predict what this possbile line of investigation with the isotopes will show. See **Radiophosphorus.**

**Resorcin Blue** (CI, 908)—fluorescent blue, iris blue—Often called Lacmoid. See Nebel, B. R., Stain Techn., 1931, **6,** 27–29.

**Resorcin-Fuchsin,** see **Weigert's** resorcin-fuchsin method for elastic fibers.

**Respiratory System.** This contains very diversified structural components for which no single technique or group of techniques can be offered. But the interpretation of the preparations depends, as in all systems of the body, on the age. A chapter by Macklin, C. C. and M. T., in Cowdry's Problem of Ageing. Baltimore: Williams and Wilkins, 1942, 936 pp. gives the necessary background and numerous hints and references to technique. See **Lungs, Trachea, Nasal Passages** and **Nasal Sinuses.**

**Reticular Fibers.** These are more finely divided and tend more to form a reticulum than the collagenic fibers. Yet there may be anatomical continuity between collagenic and reticular fibers and there is reason to believe that the two are fundamentally similar. They are not so conveniently viewed in the fresh condition because to make thin spreads is more difficult. For details see Maximow, A. A., von Mollendorf's Handbuch der Mikroskopischen Anatomie des Menschen, 1927, 2 (1), 232–583. The principal methods for reticular fibers in sections involve silver impregnation (**Perdrau, Foot, Wilder, Gomori** and **Laidlaw**), the choice of which will to some extent depend on the kind of tissue studied. There are, however, several which are stains (**Kinney's Method** and **Biebrich Scarlet** and **Picro-Anilin Blue**).

**Reticulocytes.** These are the stages recognized in the red series before the as-

sumption of properties of **Erythrocytes.** An excellent review of the properties of reticulocytes is supplied by Orten, J. M., Yale J. Biol. & Med., 1933–34, **6**, 519–539. Reticulocytes can easily be identified by supravital staining with brilliant cresyl blue. First make a thin film of the dye on slide by allowing a 1% solution in absolute alcohol, spread evenly, to evaporate. Then mount fresh blood, ring with vaseline and observe. To make relatively permanent specimens, remove the cover glass after 2 min., smear dry and color by **Wright's Stain.** The supravital staining with cresyl blue is inhibited by certain substances (Heath, C. W. and Daland, G. A., Arch. Int. Med., 1931, **48**, 133–145). For a calculation of experimental error in reticulocyte counts, see Marcussen, P. V., Folia Haemat., 1938–39, **61**, 49–64 and for *fragility tests*, see Mermod, C. and Dock, W., Arch. Int. Med., 1935, **55**, 52–60. Resistance to hypotonic sodium chloride solutions is described by Daland, G. A., and Zetzel, L., Am. J. Med. Sci., 1936, **191**, 467–474. The *protoporphyrin* content of reticulocytes can be estimated by the fluorescence technique. Watson and Clarke (C. J. and W. O., Proc. Soc. Exp. Biol. & Med., 1937, **36**, 65–70) have discovered that it is greater than in erythrocytes and that brilliant cresyl blue is precipitated by protoporphyrin which may explain the characteristic staining of reticulocytes by this dye.

**Reticulo-Endothelial Blockade.** Supposed to be a method whereby R. E. cells are so blocked by the ingestion of one foreign material that they are unable to take in another. For experiments with India ink and brilliant vital red and critical statement, *see* Victor, J., Van Buren, J. R., and Smith, H. P., J. Exper. Med., 1930, **51**, 531–548.

**Reticulo-Endothelial System.** This is by definition made up of the reticular cells of the connective tissues plus certain special endothelial cells chiefly located in the spleen, liver, bone marrow, adrenals and lymph nodes. All have the common property of phagocytosing particulate matter such as trypan blue, carbon, etc. These, and possibly others, may leave their moorings and become free cells when they become known as **Monocytes** or **Macrophages.** A better term is the "system of macrophages" (or big eaters) in which emphasis is placed on function not origin. See **Vital Staining.**

**Retina,** see **Eyes.**

**Retterer's Stain** for muscle. Fix in 10 parts 80% alcohol plus 1 part formic acid. Stain paraffin sections with alum carmine. Muscle light red, all connective tissue unstained.

**Rhodamine B** (CI, 749)—brilliant pink B, rhodamine O—A basic xanthene dye. It gives a good color contrast with methylene blue in coloration of the spleen (Houcke, E., C. Rend. Soc. de Biol., 1928, **99**, 788–789).

**Rhodamine O,** see **Rhodamine B.**

**Rhodamines.** Similar in some respects to pyronins but there is a third benzene ring affixed to central carbon atom and to this in turn is attached a carboxyl in ortho position. Examples: Rhodamine B and fast acid blue R. Rhodamin B (Merck) and 6G IG. have been employed as vital stains. When used with plant cells mitochondria become fluorescent (Strugger, S., Protoplasma, 1938, **30**, 85–100).

**Rhodopsin** (G. *rhodon*, rose + *ops*, eye). Visual purple present in external segment of the rod cells of retina (See Arey in Cowdry's Special Cytology, 1932, **3**, 1211–1291).

**Riboflavin** (lactoflavin) shows typical green fluorescence in living liver and kidney observed under fluorescence microscope (Ellinger, P., and Koschara, W., Ber. deutsch. Chem. Ges., 1933, **66**, 315–317, 808–813, 1411–1414).

**Ribonuclease** (Yeast thymonucleic acid), occurrence (Greenstein, J. P. and Jenrette, W. V., J. Nat. Cancer Inst., 1941, **2**, 301).

**Rickettsia** are small, gram negative, bacteria-like organisms which are insect transmitted and typically inhabit endothelial cells of vertebrate hosts named after H. T. Ricketts who died of typhus fever while investigating them. They are best stained by **Giemsa's** method after fixation in Zenker's, Bouin's or Regaud's fluids..

1. *Rapid staining with thionin.* Make sat. sol. of thionin in aq. dest. Precipitate by adding 10% NaOH. Collect ppt. on filter and wash until filtrate becomes neutral. Dissolve ppt. in 2% phenol. Stain absolute alcohol fixed smears only 30–50 sec. Drain, wash quickly in absolute alcohol, clear in xylol and mount in cedar oil. Rickettsia, deep violet; cytoplasm, light violet; red cells bluish green (Laigret, J. and Auburtin, P., Bull. Soc. Path. exat., 1938, **31**, 790–791).

2. *Fuchsin staining method.* Smear tissue culture on slide. Dry in air, then by heat. Filter directly on to smear 0.25% basic fuchsin in phosphate solution buffered to pH 7.4 or in aq. dest. made pH 7.2–7.4 by adding sodium hydrate or carbonate (see **Buffers**). Stain 4 min. Wash quickly with 0.5% aq. citric acid. Pour off citric and wash

rapidly in tap water. Counterstain in 1% aq. methylene blue, 10 sec. Rickettsia, red; cells, blue; not recommended for tissue sections (Zinsser, H., Fitzpatrick, F. and Hsi Wei, J. Exp. Med., 1939, **69**, 179–190). This is very similar to Machiavello's method described by Cox (H. R., Publ. Health Rep., 1939, **53**, 2241–2247) as superior to Giemsa's stain for Rickettsiae of Rocky Mt. Spotted Fever and Typhus groups.

**Ringer** solution. NaCl, 0.85 gm.; KCl, 0.025 gm.; $CaCl_2$, 0.03 gm.; aq. dest., 100 cc. Lee (p. 731) advises for amphibians same except that NaCl is 0.65 gm. and $NaHCO_3$, 0.02 gm. is added to make pH about 7.0–7.4. If $NaHCO_3$ is present it must not be sterilized by heat.

**Ringer-Locke** solution. NaCl, 0.85 gm.; KCl, 0.042 gm.; $CaCl_2$, 0.025 gm.; $NaHCO_3$, 0.02 gm.; aq. dest., 100 cc. for cold blooded animals. Lee (p. 73) advises same except that NaCl is 0.65 gm. Should be freshly made. Owing to presence of $NaHCO_3$ must not be sterilized by heat.

**Romanowsky Stains** contain polychrome methylene blue eosinates. Those of Wright, Leishman and Wilson are well-known. The Romanowsky effect is the lavender-red coloration by them of the nuclei of lymphocytes, monocytes, protozoa and other materials. Acetone solvents for Romanowsky stains (Kingsley, D. M., J. Lab. & Clin. Med., 1936–37, **22**, 524–531). Polychroming process (*ibid*, 736–752). Dyes for (*ibid*, 1264–1273). Large bibilographies.

**Romieu Reaction** for proteins. Fix in formalin, in alcohol or in Bouin's fluid. Make rather thick sections in paraffin or preferably in celloidin. Cover section with a drop of syrupy phosphoric acid. After few minutes in oven at 56°C. examine directly. A red or violet color develops in location of proteins. According to Blauchetière and Romieu (A. and B., C. Rend. Soc. de Biol., 1931, **107**, 1127) it is due to the tryptophane grouping. See Lison, p. 129.

**Rongalite White,** said to stain normal but not cancerous cells (Roskin, G., Bull. d'Hist. appl., 1938, **15**, 20–23).

**Rosanilin** (Magenta I) is triamino-tolyl-diphenyl-methane chloride, a component of most **Basic Fuchsins.** Rosanilin with methylene blue for Negri bodies (Schleifstein, J., Am. J. Pub. Health., 1937, 27, 1283–1285).

**Rosazine,** see **Azocarmine G.**

**Rose Bengal** (CI, 779). A xanthene dye of fine color used for several purposes including the staining of **Soil Bacteria** by Conn (p. 157). Make suspension of soil in 9 times its weight of 0.015% aq. gelatin. Spread drop on clean slide and dry over boiling water bath. Cover, while still on bath for 1 min., with rose bengal 1 gm.; $CaCl_2$, 0.01 gm.; 5% aq. phenol, 100 cc. Wash quickly in water. Dry and examine. See **Eosins.**

**Rosin** U.S.P. XI (colophony, yellow resin, abietic anhydride) used in Grieves' method for undecalcified dental tissues and bone.

**Rosinduline GXF,** see **Azocarmine G.**

**Rosophenine 10B,** see **Thiazine Red R.**

**Rouget Cells,** see Pericapillary cells.

**Rubber Paraffin.** Johnson (J., Applied Micr., 1903, **6**, 2662) has recommended 1% crude India rubber in paraffin colored amber yellow by addition of asphalt heated to 100°C. 1–2 days. The supernatant fluid is poured off and used as ordinary paraffin. **Double Imbedding** in celloidin and paraffin has been suggested. See Beyer (E. M., Am. J. Clin. Path., Tech. Suppl., 1938, **2**, 173–175).

**Russell-Body Cells,** Russell bodies and the cytoplasm of plasma cells are probably not hemoglobiniferous because they do not react as do the substances in known hemoglobiniferous cells with reference to isoelectric point of hemoglobin (Kindred, J. E., Stain Techn., 1935, **10**, 7–20).

**Ruthenium Red** is ammoniated ruthenium oxychloride, a mineral pigment. Conn (p. 187) says that it is used microscopically as a test for **Pectin** for which some consider it to be specific.

**Ruthenium Tetroxide,** as a fixative said to be superior in some ways to osmium tetroxide; but it decomposes readily and penetrates poorly. To prevent decomposition make 1% sol. in sat. chlorine water (Carpenter, D. C. and Nebel, B. R., Science, 1931, **74**, 154–155).

**Saffrosin,** see **Eosin B** or bluish.

**Safranin.** In the safranins one nitrogen of the azin group is pentavalent and to this a benzene ring is attached. All are strongly basic. Amethyst violet, azocarmine G, Magdala red, phenosafranin and safranin O are mentioned.

**Safranin Acid Violet,** see **Neutral Safranin.**

**Safranin B Extra,** see **Phenosafranin.**

**Safranin O** (CI, 841)—cotton red, Gossypimine, safranin Y or A—Commission Certified. A basic azin dye of great usefulness which is sold as a mixture of di-methyl and tri-methyl phenosafranins. Conn (p. 97) explains that the shade depends upon their relative proportion. The red is deeper when there is more of the latter. Safranin O

can be employed irrespective of whether *safranin O wasserlöslich*, or *safranin spiritlöslich* or *safranin gelb* is called for. The *safranin pur*, likewise of Grubler and Co., is in his opinion methylene violet (CI, 842). Safranin O is one of the finest nuclear stains especially in the **Safranin Light Green** method. It is also useful in making certain neutral stains (**Neutral Safranin**). Standardized technique for safranin O employing buffered solutions is given by Sawyer, C. H., Stain Techn., 1940, **15**, 3–7.

**Safranin Y** or **A**, see **Safranin O.**

**Safranin-Gentian Violet-Orange G.** This is Flemming's tricolor stain for nuclei. As described by the Bensleys (p. 88). Fix in Flemming's fluid and bring paraffin sections down to 95% alcohol. Stain in equal parts sat. safranin in 95% alcohol and filtered sat. anilin oil in aq. dest., 2–24 hrs. Rinse in aq. dest. and stain in sat. aq. gentian violet (crystal violet), ½–2 hrs. Drop on sat. aq. orange G, 30–60 sec. Drop 95% alcohol on slide until clouds of color cease coming off. Drop on clove oil and differentiate under microscope. Clear in benzol and mount in balsam. Violet should color diffused chromatin strand; safranin denser part; and orange G, the background.

**Safranin-Light Green.** Stain sections 24 hrs. in 2% aq. safranin O and wash out the excess safranin in 0.25% aq. light green (acid violet). Chromatin appears red and acidophilic nuclear inclusions caused by viruses green. A very brilliant stain but the green fades in the course of a month or two. Standardized safranin O technique advised by C. H Sawyer (Stain Techn., 1940, **15**, 3–7) is: overstain deparaffinized sections in 0.1% light green S.F. or fast green FCF in 50% alcohol adjusted to pH 2.4 with 0.1 *N* HCl for 4 hrs. or more. Destain in Sörensen's buffer pH 8, 30 minutes or more. Overstain in 0.1% aq. safranin O at least 4 hrs. Rinse in aq. dest. Destain in 0.01 *N* HCl (pH 2) or in 0.001 *N* HCl (pH 3) for light green and fast green respectively, 15 min. After rinsing in aq. dest. dehydrate in 2 changes dioxan, pass through xylol and mount in balsam. As fixatives Sawyer finds **Petrunkevitch's** paranitrophenol-cupric-nitrate-nitric and picro-formol-acetic better than Bouin's fluid. Zenker's fluid can be employed.

**Sandarac** mixed with dioxan, camphor and salol is recommended by McClung (p. 40) as a mounting medium in place of balsam.

**Sandison's Technique** for inserting transparent chambers in rabbit ears (Sandison, J. C., Anat. Rec., 1924, **28**, 281). This has been improved by Clark, E. R. et al., Anat. Rec., 1930, **47**, 187–211 and by Abell, R. G. and Clark, E. R., Anat. Rec., 1932, **53**, 121–140. See modifications by Williams, R. G., Anat. Rec., 1934, **60**, 487–491 and by the same author (*ibid*, 493–499) the latter for insertion into skin. Moore, R. L., Anat. Rec., 1935–36, **64**, 387–403) has adapted the chamber for insertion into dog's ear.

**Sarcolemma.** Special technique for, see Dahlgren in McClung (p. 132).

**Scarlet B** or **EC**, see **Biebrich Scarlet,** water soluble.

**Scarlet B Fat Soluble,** see **Sudan III.**

**Scarlet J, JJ, V,** see **Eosin B** or bluish.

**Scarlet R,** see **Ponceau 2R.**

**Scarlet Red,** see **Sudan IV.**

**Schaudinn's Fixative.** Sat. mercuric chloride in 0.85% aq. sodium chloride 2 parts. Add 1 part 95% ethyl alcohol and enough glacial acetic to make 1% solution immediately before use. For **Protozoa,** staining in bulk.

**Schiff's Reaction** for aldehydes (Bourne, p. 22) is basis of Feulgen reaction for **Thymonucleic Acid.**

**Schneider's Aceto-Carmine,** see **Aceto-Carmine.**

**Schultz, H.** Cholesterol Test. Cut frozen sections of formol fixed material. Place sections in a 2.5% solution of iron alum mordanting for 3 days in low temperature (37°) oven. Rinse sections in distilled water and mount on slides. Blot dry with filter paper. Add a few drops of a mixture of equal parts of concentrated sulphuric acid and glacial acetic acid. The appearance of a blue-green color indicates that cholesterol, either in free or ester form, was present in the sections before treatment. Both acids must be of analytical reagent standard and the sulphuric acid at least 98% pure. The appearance of bubbles in large numbers indicates impure reagents. (Above details supplied by Dr. R. H. Knouff). See Knouff, R. A., Brown, J. B. and Schneider, B. M., Anat. Rec., 1941, **79**, 17–38. Swyer, G. I. M., Cancer Research, 1942, **2**, 372–375 has checked in a satisfactory way the Schultz test with quantitative determinations of cholesterol in normal and enlarged prostates.

**Schultze's Method** for clearing embryos has been modified by Miller. See **Cartilaginous Skeleton.**

**Sebaceous Glands.** Method for staining *in toto* (Badertscher, J. A., Stain Techn., 1940, **15**, 29–30). Fix fresh skin for 24 hrs. in 10% formalin, or take skin from dissecting room cadaver and fix in the same way. Make free hand vertical sections 1–2 mm. thick from region pos-

sessing the glands. Whole pieces of skin 12 mm. square or larger (without subcutaneous fat) can be used in place of the sections. Pass through 50 to 70% alcohol. Stain for 12–24 hrs. in a mixture of 70 parts absolute ethyl alcohol, 20 parts 10% aq. sodium hydroxide and 10 parts of aq. dest. saturated with Sudan IV. Wash away excess stain by repeated changes of 70% alcohol until glands become sharply outlined. Clear in glycerin. Mount in Brandt's glycerin jelly (melted gelatin, 1 part; glycerin, 1½ parts + few drops carbolic acid). Glands scarlet in transparent background. This method may prove useful to bring out the distribution, number, size and other features of sebaceous glands in different conditions as well as at different ages. The same method can be used for Meibomian (tarsal) glands after a little preliminary dissection described by the author.

Another method of staining sebaceous glands *in toto* employed in the Barnard Free Skin and Cancer Hospital is to separate epidermis from dermis by the dilute acetic acid method (see **Epidermis**) and stain the epidermal sheet, with sebaceous glands attached, with sudan III or IV as one would a section for fat. A hematoxylin counterstain is useful.

The technique of **Fluorescence Microscopy** is useful. Figge, F. H. J., Bull. School of Med. Univ. Maryland, 1942, **26**, 165–176 has described the remarkable red, white or yellow fluorescence of blackheads which is characteristic of different individuals.

**Secretion** contrasted with excretion (Cowdry's Histology, p. 259).

**Sectioning,** see **Celloidin, Frozen, Gelatin** and **Paraffin Sections.** Also **Bone** grinding and **Teeth** cutting with power lathe.

**Selenium.** Intravenous injections of colloidal solutions of selenium in rabbits are described by Duhamel, B. G., C. rend. Soc. de Biol., 1919, **82**, 724–726. See **Radioselenium.**

**Semen Stains,** examination of for spermatozoa. Place piece of soiled cloth not more than ½ inch in diameter on a slide. Add few drops saline solution and scrape surface of cloth with blunt edge of a scalpel. Carry scrapings off with fluid and spread on a slide. Dry and fix with heat. Cover with 4 cc. 1% aq. Wollschwartz (Grubler) + 0.05 cc. 2% aq. sulphuric acid, 5 min. Wash in water. Counterstain 6–8 sec. with Loeffler's methylene blue diluted with 15 parts aq. dest. Wash in aq. dest., dry and examine. Heads of spermatozoa bright golden or yellowish color, all else gray. Useful in legal medicine (Williams, W. W., J. Lab. & Clin. Med., 1936–37, **22**, 1173–1175). See author's figures.

**Seminal Fluid.** To study in sections centrifuge fluid ½ to 1 hr. after ejaculation for 20 min. at 3000 r.p.m. Fix centrifugate in 4% formalin, 48 hrs. 2 changes. Take sediment into abs. alc., then 9 parts abs. and 1 part xylol. Gradually increase xylol to 9 parts to 1 part alc. Xylol paraffin 30 min. Then 54°C. melting paraffin for 3 hrs. in incubator at 58°C. After 3 hrs. in 60°C. melting paraffin embed and section 2–3 microns thick (Joël, K.. J. Lab. & Clin. Med., 1939, **24**, 970–972).

**Sense Organs,** see **Eyes, Ear, Pacinian Corpuscles, Meissner's Corpuscles, Krause's End Bulbs, Nerve Endings.**

**Sensitol Red,** see **Pinacyanol.**

**Sharpening,** see **Microtome Knife.**

**Shrinkage** caused by fixation, dehydration and clearing of nervous tissues has been measured by King, H. D., Anat. Rec., 1910, **4**, 213–244 and by Allen, Ezra, Anat. Rec., 1916, **10**, 565–589.

**Sickle-Cell Trait.** A critical study of methods for detection by Diggs and Pettit (L. W. and V. D., J. Lab. & Clin. Med., 1939, **25**, 1106–1111) gives first place to the *Moist Stasis* technique of Scriver and Waugh. Place a rubber band about proximal part of a finger. Leave 5 min. Puncture and examine fresh blood for sickle cells. According to Hansen-Pruss (O. C., J. Lab. & Clin. Med., 1936–37, **22**, 311–315) the maximum percentage of sickle cells is produced in 4–5 hrs. by supravital staining with brilliant cresyl blue or janus green, while it takes 24 hrs. in unstained moist preparations.

**Siena Orange** (K. Hollborn, Leipsig) = sodium paradipicrylamine, an alleged stain for potassium (Carere-Comes, O., Zeit. wiss. Mikr., 1938, **55**, 1–6).

**Silicon.** Easily recognizable in sections viewed in polarized light. It often occurs as sericite in combination with magnesium, iron and other minerals, see Jones, W. R., J. Hyg., 1933, **33**, 307–329. Microtechnique is discussed by Policard, A., and Mastin, E., Bull. d'Hist. Appl., 1933, **10**, 22–36. Microincineration is useful but Scott says that an exaggerated idea of amount may be obtained (McClung, p. 659).

**Silver** is occasionally found in the tissues particularly after treatment with silver nitrate or argyrol. It appears as brown to black granules or masses, is definitely blackened by ammonium sulphide and may be removed by a mixture of sodium thiosulphate and potassium ferricyanide solutions. Recently a method

based on reaction between silver and *p*-dimethylaminobenzylidenrhodanin has been described and illustrated in colors (Okamoto, K., Utamura, M. and Akagi, T., Acta Scholae Med. Univ. Imp. in Kioto, 1939, 22, 361–372).

**Silver Chloride Dichlorfluoresceinate** coloration of vascular endothelial cells (Bensley, R. D. and S. H., Anat. Rec., 1935, 64, 46–49). Inject intravenously 0.8% aq. dichlorfluorescein until animal becomes quite yellow. Kill animal; remove tissues and immerse in 10% aq. silver nitrate or in Bensley's **Silver Citrate** solution until salmon pink color develops. Fix in 10% neutral formalin. Dehydrate in alcohol and **Iso-Safrol,** clear in iso-safrol and mount in balsam. Endothelial cells outlined in pink. On exposure to light color changes in time the silver becoming brown and black. See demonstration of **Chlorides** in lungs by this method.

**Silver Citrate** injection of blood vessels (Bensley, R. D., Am. J. Anat., 1929, **40,** 146–169). This method has proved of great value in the investigation of efferent vessels of renal glomeruli. It can be employed to advantage in other situations particularly in association with supravital staining of **Pericapillary Cells** with janus green. To make up the solution dissolve 4 gm. silver nitrate in 100 cc. aq. dest. and remove to dark room. Completely precipitate silver as silver phosphate by addition of sodium phosphate solution. Wash ppt. repeatedly with aq. dest. decanting supernatant fluid. Make up to volume approximately 30 cc. Dissolve ppt. by adding 28 gms. pure citric acid (or tartaric acid) in crystals. Dilute with aq. dest. to 1000 cc. and keep in dark.

For use, dilute this stock solution with 3 times its volume 1% aq. sodium citrate. Kill the animal by bleeding. For kidneys and other abdominal viscera insert into aorta cannula connected by rubber tubing with pressure bottle. First perfuse with 1% aq. sodium citrate with the pressure bottle about 60 cm. above cannula. When clear fluid, free from blood, appears in inferior vena cava, clamp tube and replace citrate solution with silver solution. Raise bottle about 150 cm. above cannula and release clamp. Determine length of time of perfusion by trials. When complete, immediately make frozen sections to determine results and fix other pieces in 10% formalin for 24 hrs. Cut paraffin sections desired thickness. Mount them in usual way, run down to water and develop in light in diluted photographic developer or simply by direct exposure to sunlight or arc light. Counterstain in **Mayer's Acid Carmine,** hematoxylin, acridine red or some other suitable dye. Dehydrate, clear, mount in balsam.

**Silver Gray,** see **Nigrosin,** water soluble.

**Silver Methods.** General statement. A brief historical review by Silver, M. L., Anat. Rec., 1942, 82, 507–529 shows that progress has been made in the control of these techniques to the point where they yield reliable results with considerable uniformity. Impregnation of blocks of tissue and reduction of the silver in various ways were and still are the bases of the methods of **Golgi, Cajal** and **Bielchowsky** which have contributed so much to our knowledge of the **Nervous System,** which see. But one had to wait until the sections were cut and examined to ascertain the results. Sometimes they were all that heart could desire; at other times the worker faced repeated disappointments. Having labored with the silver impregnation of neurofibrils I have always avoided silver methods whenever others can be employed in their place. Now however with the successful application of reduced silver to sections mounted on slides the technique is brought from the insides of the blocks of tissue which one cannot see into the open, thanks to Rogers, W. M., Pappenheimer A. M., and Goettsch, M., J. Exp. Med. 1931, 54, 167–169. Another advance was the introduction of protargol as the silver salt for treating sections of the central nervous system by Bartelmez, G. W. and Hoerr, N. L., J. Comp. Neurol., 1933, 57, 401–428. Then, likewise in Bensley's laboratory, Bodian, D., Anat. Rec., 1936, 65, 89–97 employed protargol with hydroquinone as reducer and speeding up results by copper, mercury and acid. Finally Davenport, H. A., McArthur, J., and Bruesch, S. P., Stain Techn., 1939, 14, 21–26 dispense with copper, and, by combining protargol and silver nitrate at optimum pH, reduce staining time of sections of peripheral nerves to 2 hrs. In addition, Silver (*loc. cit.*) by well planned experiments has shown that staining with silver is brought about through adsorption and flocculation of electrically charged silver micelles by suitably charged surfaces. When these newer methods are widely brought to bear upon tissues of the body in normal and pathological conditions a significant service will be performed. Suffice it here to give a few details under **Nervous System, Spirochetes,** tests for **Calcium, Chloride, Vitamin C, Reticular Fibers, Melanin,** etc.

**Silver Staining** of bone (McCollum, E. V.,

Simmonds, N., Shipley, P. G. and Park, E. A., J. Biol. Chem., 1922, **51**, 41–49).

**Silver's** rapid silver-on-the-slide method for nervous tissue (Silver, M. L., Stain Techn., 1942, **17**, 123–127). A new feature of this technique is the reducing solution.

1. For *nuclei, fine fibers* and *nerve terminals*, fix with 10% neutral or commercial formalin in 1% aq. sodium chloride with Bouin's fluid or with some other fixatives which he specifies preferably by **Perfusion.**

Cut frozen sections 10–40$\mu$ or dehydrate slowly, imbed in paraffin or celloidin and cut 2–20$\mu$. Mount paraffin sections on slides and deparaffinize in the usual way. In the case of celloidin sections remove celloidin with several changes acetone and of equal parts absolute alcohol and ether and pass down through alcohols to water.

To make reducing solution dissolve 64 gm. Rochelle salts (potassium sodium tartrate) in 500 cc. aq. dest. Boil vigorously. Add 10 cc. 10% aq. silver nitrate and boil again at least 5 min. Remove from flame. Add 0.3 gm. crystalline magnesium sulphate and while simmering 0.2 gm. $K_2S$ (U.S.P.) employing only the brown unoxidized part of 1 piece. Filter while hot and make up filtrate with aq. dest. to 4 liters. This reducer improves slightly with age.

Place mounted paraffin sections or frozen or celloidin sections in equal parts above reducer and 0.5% aq. protargol (Winthrop Chemical Co., Inc., New York) at 45–55°C. Staining is progressive and ordinarily takes 2–3 hrs. Remove and examine. When complete, generally before a grossly visible reduction of silver is evident in the solution, remove, wash in 2 changes aq. dest., dehydrate, clear and mount. More finely myelinated fibers are revealed than are demonstrated by the standard Weigert technique.

2. For *myelin sheaths* and *mitochondria* fix with 10% formalin in 1% aq. potassium bichromate or with 10% formalin in 1% aq. NaCl again preferably by perfusion, and mordant small blocks of the tissue in 3% aq. potassium bichromate for 7 days (This mordanting can be dispensed with if tissue is in fixative for more than 1 week.). Wash, dehydrate, imbed (in paraffin), cut 4–20$\mu$ and mount on slide. Remove imbedding medium and proceed as described above.

**Sinusoids** are capillaries of large diameter through which the circulation is slower. The endothelial cells of their walls ingest some forms of particulate matter in the blood stream. The best place to demonstrate them is in carmine stained sections of formalin fixed liver of an animal injected intravenously with India ink as described under **Vital Staining.**

**Sizes of Organs.** See **Normals.**

**Skin.** No other part of the body is similarly spread out for examination *in vivo*. Much is to be gained by correlation of gross and microscopic study. Alterations in color, moisture, consistency and thickness can easily be detected. Changes in sensitivity and in the number and activity of sweat glands can be determined by appropriate methods. Simple techniques are available for the visualization of **Lymphatic Vessels,** and the **Capillaries** in the dermal papillae can be demonstrated microscopically and their behavior recorded in moving pictures. See Thomas Lewis' classic, The Vessels of the Human Skin and their Responses. London: Shaw & Sons, 1927, 322 pp. Very important is direct study of the skin with hand lens or binocular microscope.

But examination in sections will always remain the basic method of study. Hair, where present, should be cut short with scissors and removed with an electric razor, an instrument which does not require the use of any soap and does not scrape away the surface. Samples of skin removed at autopsy are satisfactory for some purposes up to about 24 hrs. if the body has been kept cool because autolytic changes take place comparatively slowly in the skin. But biopsy specimens are much better. The local anesthetic should be injected in a circle about the skin to be excised and the observer should be on the lookout for slight modifications if the sections include the actual area into which it is forced. Obviously the specimen should be lifted, never pinched with forceps.

Because the skin is made up of 2 tissues, avascular epidermis and vascular dermis, closely bound together, differential shrinkage is a troublesome factor. Evans, R., Cowdry, E. V. and Nielson, P. E., have found in this laboratory that, owing to shrinkage or drawing together of the dermis, the folds in the epidermis are accentuated to an extent much greater than is generally realized. This is more marked in young skins than in those of old people and in living skin than in skin excised after long delayed autopsy. It is apparently not feasible to entirely side step this kind of artefact but the tendency of the whole specimen to curl up can be obviated by spreading it out with dermis down on a piece of

wooden tongue depressor or stiff cardboard for the first few minutes of fixation. If interest definitely centers in the dermis it should be mounted with epidermis down. But it should not be kept in either position too long because the complete entry of fixative will thereby be prevented. After 3 or 4 hrs. the specimen should be trimmed with a new wet razor blade.

Frozen sections are essential for rapid diagnosis, for staining with **Sudan** and for many other purposes. The technique most used by dermatologists is to fix in **Bouin's Fluid** and to stain paraffin sections with **Hematoxylin** and **Eosin.** After **Zenker Fixation, Mallory's Connective Tissue Stain,** or **Masson's Trichrome Stain,** is suitable for muscle and collagenic tissue. **Weigert's** resorcin fuchsin is recommended for elastic fibers. The **Dopa Reaction** is required for melanin precursors. For nerve fibers the **Bodian** method is probably the best. Another silver technique advised for the skin is that of **Jalowy.**

MacCardle, R. C., Engman, M. F., Jr. and Sr., Arch. Dermat. & Syph., 1941, **44,** 429–440 give details of spectrographic analysis of skin lesions. See also **Microincineration.** Ultracentrafugation method for determination of intranuclear viscosity (Cowdry, E. V. and Paletta, F. X., Am. J. Path., 1941, **17,** 335–357). Methods of transplantation are described by Kelly, R. W. and Loeb, L., Anat. Rec., 1939, **74,** 487–509 and of fluorescence examination by Cornbleet, T. and Popper, H., Arch. Dermat. and Syph., 1942, **46,** 59–65. An adaptation of the **Sandison** technique is recommended by Williams, R. G., Anat. Rec., 1934, **60,** 493–499. See **Sebaceous** and **Tarsal** glands, **Hairs, Nails, Feathers.**

If it is not desired to investigate a particular area, to which attention has been called by its unusual gross appearance; but, instead, to demonstrate some special component, or response, of the skin one should be guided in selection of the specimen by the location where the component or response is most likely to be found. Thus *Meissner's corpuscles* are best seen in sections of skin of palmar surface of finger tips. Weddel, G., J. Anat., 1941, **75** (3), 346–367 reports that multiple groups of **Krause's** end-bulbs occur beneath each cold spot in the forearm about 1 mm. inward from the skin surface. Many helpful clues are supplied by Lewis, T., Pain. New York: MacMillan, 1942, 192 pp. He quotes Strughold as stating that pain spots are aggregated as closely as 200 per sq. cm. in supraclavicular, antecubital, inguinal and popliteal fossae while they are rare (40–70 per sq. cm.) on tip of nose and ear, soles and palms (see **Nerve Endings**). The skin of axillary, pubic and nipple areas is more likely than that of the rest of the body to respond to sex hormones. Adjustments to external environment are to be expected in exposed parts. To search for sweat glands in those mammals which do not possess any is futile. To expect all epidermal layers in thin epidermis is likewise contraindicated.

**Fluorescence Microscopy** is capable of yielding interesting results in distinction between psoriasis and hyperkeratosis scales (Radley, J. A. and Grant, J., Fluorescence Analysis in Ultraviolet Light. New York: Van Nostrand, 1935). Further indications on fluorescence are given under **Hair** and **Sebaceous Glands.**

Now that epidermis can be conveniently separated from dermis it is desirable to give details of technique relating to it under a separate heading. See **Epidermis.**

**Skyblue** (CI, 1286)—coelestin blue, coeline, coeruleum—a mineral pigment, cobaltous stannate, seldom used in medical research.

**Slides,** see **Cleaning.**

**Slime Forming Bacteria,** Conn's method. Stain for about 1 min. with a little heat in Rose bengal 1 gm., 5% aq. phenol 100 cc., 1% aq. $CaCl_2$, 1 cc.; then wash quickly and dry (McClung, p. 146).

**Small Intestine.** Many conditions influence the appearance seen in sections. If fixed while distended with food material, the spaces between the villi are more noticeable, the villi shorter and the muscular layers thinner than when fixed while strongly contracted. See illustrations provided by Johnson, F. P., Am. J. Anat., 1912–13, **14,** 235–250 and **Contraction Bands.** The time after feeding and the character of the food has a marked influence on structure. The cytoplasmic granules of the **Paneth Cells** are almost all discharged in guinea pigs 6 hrs. after feeding. They are present in large numbers after fasting for 24 hrs. (Klein, S., Am. J. Anat., 1905–06, **5,** 315–330). Even vitamin B deficiency alters the distribution of intraepithelial fat (Mottram, J. C., Cramer, W., and Drew, A. H., Brit. J. Exp. Path., 1922, **3,** 179–181). According to Hamperl (H., Ztschr. f. Mikr.-Anat. Forsch., 1925, **2,** 506–535) **Enterochromaffin Cells** can no longer be found in humans autopsied as late as 4–5 hrs. after death. The incidence of **Contraction Bands** in muscle is increased by exposure to air

and mechanical manipulation before fixation. Villi are very prone to exhibit **Agonal Changes.** If the individual has fasted for a long time before death a marked invasion of the mucous membrane by lymphocytes is to be expected. See Fig. 158, Cowdry's Histology. It may extend throughout the gastrointestinal tract being greatest in the stomach and least in the large intestine.

A good way to examine the wall of the small intestine is to push a test tube of appropriate size into the lumen of a segment. This will hold it open and facilitate dissection. Strip off the serosa, then the tunica muscularis, noting the direction of the fibers and leaving only the mucosa. Take small pieces of mucosa and mount in physiological saline inside up and examine at low magnification. Finally with dissecting needles pick out separate villi and study with oil immersion objective. To obtain a clearer concept of individual muscle fibers first macerate the intestine on the tube in 15% aq. nitric acid for 2–3 days. Consult Carey, E. J., Anat. Rec., 1921, **21**, 189–215 and Goerttler, K., Morph. Jahrb., 1932, **69**, 329. See **Chloralhydrate Maceration.**

**Smears.** To examine fluids and tissues as thin films so that the components are individually clearly visible is often necessary. Careful preliminary cleaning of the slides is necessary. Touch the surface of a slide about 2 cm. distant from the end to a drop of blood immediately on the appearance of the latter from a puncture in the skin. Quickly apply the smooth end of another slide to the drop and the surface of the first slide so that the drop spreads along the line of contact. Then evenly push the second slide, with the blood following it, along the surface of the first slide. The angle at which the pusher is held plus the speed of smearing and the amount of blood will determine the thickness of the film. Ordinarily it should be so thin that the reds are smeared in a single layer. But for certain purposes as in the search for some parasites thick smears are the best (see **Blood Smears**).

In the case of cells in cerebrospinal and other fluids and of some bacteria and parasites it may be desirable to concentrate the objects by centrifugation because otherwise smears would show too few of them. See **Concentration Methods.** The precautions detailed above to obtain evenness are seldom required. The material simply is transferred to the slide in a platinum loop or glass pipette and spread on it. Smears of lymph nodes and spleen are generally made by drawing "streaking" the freshly cut, moist surfaces along slides. *Impression preparations* of these tissues are not smears but they serve the same purpose. In making them the surface of slide is quickly pressed against the surface of the tissue and a considerable number of the easily detachable cells adhere to the slide where they are quickly dried, or, while still wet the impression can be fixed in Helly's fluid (i.e. formalin Zenker) as advised by Maximow (see Downey, p. 2001). McClung (p. 262) recommends smears on cover glasses for certain germ cells.

The smears can be fixed by gentle heat, or by methyl alcohol or in special cases in formalin or osmic vapor. Giemsa's stain is the most popular but a great many others are available especially for **Bacteria.**

Smears cannot be made of fixed cells isolated by **Maceration** in the same way because they are not present in body fluids which when they dry facilitate sticking of the cells to the slides. It is therefore necessary to spread them on slides previously moistened with a very small amount of **Albumen-Glycerin** before drying.

**Smith-Dietrich** method for lipoids (Dietrich, A., Verh. d. Deut. Path. Ges., 1910, **14**, 263–268). Treat frozen sections of formalin fixed tissues 1–3 days in 5% aq. potassium bichromate at 37°C. After washing in aq. dest. stain 4–5 hrs. in Kultschitzky's hematoxylin (stock solution 10% in abs. alc. ripened at least 6 months, 10 cc. + 2% acetic acid, 90 cc.). Wash. Differentiate over night in Weigert's borax ferricyanide (borax, 2 gm.; potassium ferricyanide, 2.5 gm.; aq. dest., 100 cc.). Wash carefully. Mount in syrup of levulose. Lipoids dark blue. Lison (204) considers the positive staining as characteristic for a lipine (lipoid) if the possible presence of cholesterides and cholesterol is excluded.

**Smooth Muscle,** see **Contraction Bands.**

**Soap-Wax** technique for paraffin imbedding, see **Lebowich.**

**Soaps.** Sodium and potassium salts of fatty acids, see **Fischler's** modification of Benda method.

**Sodium.** A method for the retention of sodium and potassium in microincinerated tissue has been proposed by Policard, A., and Pillet, D., Bull. d'Hist. Appl., 1926, 230–235. In their opinion these two elements are present as chlorides in the tissue and their conversion to sulphates by treating the sections with sulphuric anhydride fumes makes them more stable and better able to

withstand the high temperature of incineration. See **Microincineration, Radiosodium.**

**Sodium Fluoride** effect on teeth (Cowdry's Histology, p. 267).

**Sodium Paradipicrylamine,** see **Siena Orange.**

**Soil.** Bacteria. 1. Conn's Rose Bengal method (McClung, p. 146). To 1 gm. soil add gelatin fixative (0.015% gelatin in boiling water used after it has cooled) to make 10 cc. Place about 0.01 cc. on slide to cover 1 sq. cm. Dry on boiling water bath. Stain with Rose bengal as for **Slime Bacteria.** Unless counts are to be made the amount smeared on the slide is not important.

2. Fast acid blue (C.I. 760) is strongly recommended (Romell, L. G., Stain Techn., 1934, **9**, 141–145) but it is doubtful whether any manufacturer other than I. G. Garbenindustrie makes the dye. According to the General Dyestuffs Corporation it is contained in violamin 3B. Dry suspension of soil on slide which has been fixed in alcohol with 0.05% dye in 4% aq. phenol. Washing is unnecessary. Examine smears in water. Details are given by Romell.

**Solanylin,** a dye extracted from the eggplant (*Solanum melongena*, var. *esculenta*) proposed as a substitute for hematoxylin. It will stain nuclei and mucus (Fuse and Suzuki, Arb. Anat. Inst. zu Sendai, 1935, **17**, 175–181).

**Solid Green JJO,** see **Brilliant Green.**

**Solid Green O,** see **Malachite Green.**

**Soluble Blue 3M** or **2R,** see **Anilin Blue.**

**Soluble Indulin 3B,** see **Indulin,** water soluble.

**Soluble Yellow OL,** see **Metanil Yellow.**

**Solutions.** In technique several kinds are employed.

1. *Physiological* solutions are intended to approximate as closely as possible to the tissue fluid environments of cells so that cells examined in them will not be greatly altered thereby. See **Physiological Solutions.**

2. *Normal* solutions are, on the other hand, chemical standards made by dissolving definite amounts of substance (easily calculated) in sufficient aq. dest. to make 1 liter. See **Normal Solutions.**

3. *Molar*, *molecular* and *grammolecular* solutions contain the molecular weight of the substance in grams per liter. They are of the same concentration as *normal* solutions of substances possessed of one hydrogen or other equivalent and differ from those of substances containing more than 1 such equivalent. See **Molecular Solutions.**

4. *Molal* solutions contain the molecular weight of the substance in grams + 1000 *grams* aq. dest. The designation *molal* is rarely used, *molecular* is common and *normal* most frequent.

**Sörensen's Buffers.** Sörenson's phosphate buffers are prepared from Merck's special reagents. Dry salts at 105°C. overnight and store in a dessicator over $CaCl_2$. M/15 solutions are used. To make them dissolve the following amounts in aq. dist. and make each solution up to one liter:

$Na_2HPO_4$ anhydrous..... 9.47 gm.
$KH_2PO_4$..... 9.08 gm.

To obtain a solution of the pH required, mix them in following amounts:

| pH | cc. M/15 $Na_2HPO_4$ | cc. M/15 $KH_2PO_4$ |
|---|---|---|
| 5.4 | 3.0 | 97.0 |
| 5.6 | 5.0 | 95.0 |
| 5.8 | 7.8 | 92.2 |
| 6.0 | 12.0 | 88.0 |
| 6.2 | 18.5 | 81.5 |
| 6.4 | 26.5 | 73.5 |
| 6.6 | 37.5 | 62.5 |
| 6.8 | 50.0 | 50.0 |
| 7.0 | 61.1 | 38.9 |
| 7.2 | 71.5 | 28.5 |
| 7.4 | 80.4 | 19.6 |
| 7.6 | 86.8 | 13.2 |
| 7.8 | 91.4 | 8.6 |
| 8.0 | 94.5 | 5.5 |

For range pH 8.2–9.2 see **Palitzsh Buffers.** See affect of **Phosphate Solutions** on living cells.

**Spalteholz Method** for clearing small embryos as suggested by the Bensleys. After appropriate fixation 80 and 95% alcohol 1 day each. Two changes absolute alcohol, 2 days. Equal parts benzol and absolute alcohol, 1 day. Two changes pure benzol, 1 day. Then Wintergreen oil (methyl salicylate) and benzyl benzoate by weight 5:1, 3:1 and 2:1 for very young, young and older embryos respectively (under negative pressure in vacuum pump) until cleared. Mount or store in this clearing fluid. In practice it is possible to get good results without the negative pressure. This method can be used for many tissues besides embryos. For author's account see Spalteholz, W., Ueber das Durchsichtigmachen von menschlichen und Tierischen Präparaten. Leipzig, 2nd Edition, 1914.

**Specific Gravity.** It is often desirable to ascertain the relative specific gravities of tissues, cells and parts of cells. See **Centrifugation.**

**Spectrographic Analysis,** see **Histospectrography** and **Absorption Spectra.**

**Spectrophotometric Evaluation** of blood

stains (Lillie, R. D. and Roe, M. A., Stain Techn., 1942, **17**, 57–63).

**Spermatozoa,** simple method for staining. Make smears of fresh spermatic fluid on slides and dry in air. Fix 3 minutes in 10% formalin. Stain in Harris' hematoxylin 1 minute, wash in water and dry (Fetterman, G. H., Am. J. Clin. Path., 1942, **6**, 9). Microincineration (Policard, A., Bull. d'Hist. Appl., 1933, **10**, 313–320). Helpful histochemical methods are detailed by Marza, V. B., Bull. d'hist. appl., 1931, **8**, 85–102. See **Semen.**

**Sphingomyelin,** a compound of phosphoric acid, a fatty acid, choline and sphingosine without glycerol, soluble in benzene, pyridine and hot alcohol and almost insoluble in ether, see **Lipoids.**

**Spirit Blue** (CI, 689)—anilin blue alcohol soluble, gentian blue 6B, light, Lyon and Paris blues—A mixture of di- and tri-phenyl rosanilin chlorides. Conn (p. 133) reports that it is a good stain for growing nerve fibers.

**Spirit Indulin,** see **Indulin,** spirit soluble.

**Spirit Nigrosin R,** see **Indulin,** spirit soluble.

**Spirochaetales.** The organisms of this group often require special methods for demonstration; but within the gastric glands of humans (Doenges, J. L., Arch. Path., 1939, **27**, 469–477) dogs, cats, rats and Macacus rhesus monkeys (Cowdry, E. V. and Scott, G. H., Arch. Path., 1936, **22**, 1–23) they can frequently be seen in ordinary hematoxylin and eosin preparations. Preparations of these benign organisms are therefore easily made and useful as showing intracellular forms within parietal cells. For special techniques see **Treponema Pallida.**

**Spleen.** Fixatives penetrate the spleen poorly on account of the large amount of blood in it. Consequently it is desirable to fix only thin slices of it, say 3–4 mm. thick. If the spleen is particularly soft to begin with the slices will not hold their shape and it may be necessary to cut parallel to the surface and include the capsule as a support. Direct observation of splenic venous sinuses *in vivo* (Knisely, M. H., Anat. Rec., **64**, 499–524; **65**, 23–50; MacKenzie, D. W., Whipple, A. O. and Wintersteiner, M. P., Am. J. Anat., 1941, **68**, 397–456). Transplants into omentum (Holyoke, E. A., Am. J. Anat., 1940, **66**, 87–132). For vascular injections of Malpighian bodies, see Nisimaru, Y. and Staggerda, F. R., J. Physiol., 1932, **74**, 327–337.

**Spodogramme,** term used by French histologists for the mineral skeleton of tissue revealed by **Microincineration.**

**Spore Stain,** a modification of Dorner's. Make thin film on slide. Cover with blotting paper and add freshly filtered Ziehl's carbol fuchsin. Steam 5–10 min. on hot plate, the blotting paper being moistened with the fuchsin. Decolorize instantaneously with 95% alcohol and wash in water. Add drop of sat. aq. nigrosine and spread thinly. Dry quickly and examine. Spores red, other parts of bacilli almost colorless against dark background. Said to be simpler, quicker than the unmodified Dorner's method. It is recommended for *Bacillus megatherium, B. niger, B. cereus, B. mycoides* and some cultures of *B. subtilis* (Snyder, M. A., Stain Techn., 1934, **9**, 71–72).

A modification of Schaeffer's spore stain. Support a small metal tray over asbestos centered wire gauze. Add water and heat to steaming. Slides with ends resting on either side of the tray should have droplets of water condense on their under surface. Flood properly fixed smear on slide with 5% aq. malachite green and leave in this way on steam bath 1 min. Drop in cold water, rinse thoroughly and while wet add 0.5% aq. safranin 30 sec. Rinse again in cold water. Spores, green; vegetative cells, red (Ashby, G. K., Science, 1938, **87**, 443).

**Sputum.** Amount, gross appearance, color and odor (if present) are important. Microscopic examination should first be made mounted but unstained. Look for pus, elastic tissue, pigmented heart failure cells, amebae, fungi, ova of animal parasites, colorless, hexagonal pointed Charcot-Leyden crystals, other crystalline material, etc. Stain smears by methods of **Giemsa, Gram** and for **Acid Fast** bacilli. It may be necessary to use **Concentration** methods. Interpretation of findings requires much experience.

**Staining** is the act of giving color to something. It is said to be *progressive* when the structures colored take up the stain progressively to a greater degree than do others which by contrast are not colored. Thus, in testing for iron by the Macallum method the iron is stained progressively with hematoxylin. Staining is called *regressive* when many structures are over stained and by decolorization, or differentiation, the color regresses and is retained only by those which hold it most tightly in contrast with which the others are not stained. To demonstrate Nissl bodies in nerve cells the cells are over stained with toluidin blue. By decolorization in alcohol the color is made to regress to the point where the Nissl bodies stand out colored in a cytoplasm no longer blue. See, also vital and supravital staining and acid and basic dyes.

Acid stains are often contrasted with basic ones though the dyes are usually neutral salts. In "acid" dyes it is the acid part, or anion, that is colored and does the staining; while in "basic" dye the reverse holds and it is the basic portion, or cation, that is the coloring agent. For instance, acid fuchsin is a sodium salt of sulphonic acid of fuchsin and it is the acid part which gives the color. Basic fuchsin, on the other hand, is a hydrochloride of rosanilin and it is the base, rosanilin, which stains. A "neutral" dye is a more complex association between a color acid and a color base.

Basic materials may be colored by acid dyes and acid ones by basic dyes, but this does not by any means always hold. A substance staining by an "acid" dye is said to be *acidophilic*, as for example the specific granules of eosinophile leucocytes which take the "acid" dye eosin. Similarly another material, such as nuclear chromatin is termed *basophilic* because it colors with toluidin blue which is a "basic" stain. A *neutrophilic* granule is colored by both the color acid and the color base of a neutral dye. An amphophilic one (G. *ampho*, both; *philos*, fond) will stain with either acid or basic dyes or with a neutral dye for it likes both color acids and color bases. Heterophile leucocytes (G. *heteros*, other, and *philos*, fond) possess granules which are homologous for the several species but differ in staining reaction for the species (Maximow—Bloom, Histology, 2nd Edit. 1934).

**Starch Grains.** The usual microchemical test is to color blue with dilute iodine. Starch grains can also be stained side by side with mitochondria in plant cells (Pea roots, Elodea, etc.). After **Regaud** fixation stain sections with warmed anilin fuchsin about 5 min. Differentiate in 5% alcoholic aurantia. Wash in aq. dest. Mordant in 2% aq. Tannin, 20 min. Wash in aq. dest. and stain in 1% aq. toluidin blue, gentian violet or methyl green, 5–10 min. Milovidov, (P. F., Arch. d'Anat. Micr., 1928, **24**, 8–18). Differentiate in 95% alc. dehydrate in abs. alc., clear in xylol and mount. Mitochondria red, starch blue, violet or green. Well shown in an excellent colored plate.

**Starch Paste,** as substitute for albumin-glycerin mixture in mounting paraffin sections. Mix thoroughly 1 gm. powdered starch in 10 cc. cold water. Pour into 20 cc. boiling water. Add 2 drops dilute HCl and boil 5 min. constantly stirring to free opalescent sol. from lumps of starch. Add crystal of thymol after paste has cooled. Use as the albumin mixture. Said to have advantage that it does not stain with dyes and in silver preparations. It is clean and easily made (McDowell, A. M. and Vassos, A. A., Jr., Arch. Path., 1940, **29**, 432–434).

**Steel Gray,** see **Nigrosin,** water soluble.

**Stomach,** secretory cells of. Use **Mucicarmine** or **Mucihematein** for surface epithelial cells and neck chief cells; Bensley's **Neutral Gentian** for body chief cells and any combination of dyes including a strongly "acid" stain like eosin for the parietal cells, all after Bensley's alcoholic chrome neblimate fixation. The parietal cells can be sharply stained by supravital intravascular injection with **Neutral** red or **Naphthol Blue R.** The canaliculi of the parietal cells can be impregnated with silver by a modified Golgi method (Plenk, H., von Mollendor'f Handb. d. Mikr. Anat. d. Menschen. 1932, **5**, (2), 235–402). To observe the cytological changes after discharge of strongly acid gastric juice and of juice rich in pepsin inject histamine and stimulate the vagus respectively (Bowie, D. J., and Voneberg, A. M., Quart. J. Exper. Physiol., 1935 **25**, 247–257). For mitochondria inject **Janus Green** intravascularly or fix in Regaud's fluid, mordant in potassium bichromate and stain with **Anilin-Fuchsin Methyl Green.** See localization of **Pepsin.**

**Stools,** see **Feces.**

**Storage** of specimens whether microscopic slides, paraffin or celloidin blocks or simply in preservative fluids should be systematic in all laboratories. Every specimen coming in for examination should be given an *accession number* and the data about it should be inscribed in a book. A book is better than a series of cards because cards can be removed by irresponsible persons and lost. The number, and other necessary information, should be written on the slide with a diamond pencil. This is usually done in pathological laboratories where there is much routine to be attended to. It is equally important in other laboratories devoted primarily to teaching and research even when a number of independent investigators are involved. System pays; lack of a unified system serving several people means loss and waste of valuable material.

**Striated Muscle,** glycogen distribution (Gendre, H., Bull. d'Hist. Appl., 1938, **15**, 265–276). Effect of different dehydration and clearing agents (Ralph, P., Stain Techn., 1938, **13**, 8–15). Methods for study of wave mechanics in living state (Carey, E. J., Zeit, W. and Massopust, L., Am. J. Anat., 1942, **70**, 119–133.

**Styrax,** a very highly refractile mounting medium seldom employed in histology (Lee, p. 228).

**Subcutaneous Tissue** spreads. Making (McClung's Microscopical Technique p. 336).

**Sublimate Acetic** is a fixative of which the usual composition is 95 parts sat. aq. mercuric chloride plus 5 parts glacial acetic acid. See **Laidlaw's** method for inclusion bodies. When the saturated solution of mercuric chloride is made in 95% alcohol the fixative should be called **Sublimate Alcohol Acetic.** See **Mercuric Chloride.**

**Submaxillary Glands.** These can be nicely stained by the supravital methods employed for the **Pancreas.** Stains for **Zymogen** and for **Mucus** are useful. The duct cells are the principal sites of action of the salivary gland virus when this plays an inapparent rôle. The tremendously enlarged duct cells provided with **Nuclear Inclusions** are often seen in the guinea pig's submaxillary and in several other species, see Cowdry, E. V. and Scott, G. H., Am. J. Path., 1935, **11,** 647–657.

**Submicroscopic Structure** of cytoplasm, methods and results (Frey-Wyssling, A., J. Roy. Micr. Soc., 1940, **60,** 128–139).

**Sudan II,** see **Oil Red O.**

**Sudan III** (CI, 248)—cerasin red, fat ponceau G, oil red AS, O, B or 3B, scarlet B fat soluble, Sudan G, Tony red—A weakly acid dis-azo dye, the most popular of fat stains in alcoholic solution. A sat. sol. in 70% alcohol is used in the same manner as **Sudan IV** in Herxheimer's solution (see below). *Variations* in action of sudan stains depending on character of fat and kind of fixation (Black, C. E., J. Lab. & Clin. Med., 1937–38, **23,** 1027–1036).

Staining in aqueous phase (Dufrenoy, J., Stain Techn., 1937, **12,** 71–72). Make concentrated solution of Sudan III in 5 cc. methylal (dimethyloxymethane). Add 10–20 cc. aq. dest. The mixture separates into 2 layers: the lower made up of water, methylal and Sudan III and the upper of methylal, Sudan III and water. Whether sections float or sink they take up Sudan III. See **Bell's Method** for staining fats mobilized by heat.

**Sudan IV** (CI, 258)—cerotine ponceau 3B, fat ponceau, fat ponceau R or LB, oil red IV, scarlet red—A weakly acid dis-azo dye also widely used as fat stain sometimes under heading of Scharlach R, especially in **Herxheimer's Solution.** Place frozen sections of formalin fixed tissue in 70% alcohol for a few sec. Transfer to Herxheimer's solution for 2–5 min. in a covered container to reduce evaporation and precipitation. Rinse in 70% alcohol. Wash quickly in aq. dest. Counterstain with Harris' hematoxylin. Wash in tap water. Mount in **Glycerin.** Seal with paraffin, or, if permanency is desired, with **Duco** or **Kronig's** cement. As a rule these fat stains do not last more than a few months.

**Sudan Black B.** This dye is of English manufacture and is not available in U.S. during the war. Its identity is still uncertain.

1. For *fat.* Fix tissues 24 hrs. in 5% formalin in 0.9% saline or in Zweibaum's fluid. The latter is made by adding 1 part of 2% aq. osmic acid to 7 parts of a mixture consisting of 3% potassium bichromate 6 cc.; 2% chromic acid, 3 cc.; and aq. dest. 5 cc. Wash in running water 24 hrs. In case tissue is delicate and requires support embed in gelatin before cutting frozen sections: 12.5% gelatin in 1% aq. phenol filtered, 37°C., 24 hrs. 25% solution, same. Embed in fresh 25% aq. gelatin, cool, trim, harden in 5% formalin 24 hrs. Cut frozen sections, whether first embedded in gelatin or not, 5–10 microns thick. Transfer to aq. dest. and then into 50% diacetin agitated 30 sec. To make stain, add excess Sudan Black B (I.G.F.) to equal volumes of diacetin and aq. dest., incubate at 55°C. for 2 days. Cool. Before use filter off amount required. Stain 15 micron sections 2 hrs. If speed is necessary warm in paraffin oven. 50% diacetin 30 sec. Counterstain with carmalum. Place in dish of water with care making sections "spin on surface and flatten." Float on to slide and mount in Apathy's medium. Nuclei red, lipids including myelin black (Leach, E. H., J. Path. & Bact., 1938, **47,** 635–637). Diacetin is glycerol diacetate introduced as solvent for scharlach R by Gross (W., Zeit., wiss. Mikr., 1930, **47,** 64). Since Leach does not specify what Apathy's medium is, it is suggested that temporary mounts be made in glycerin.

2. For *myelin* (Lison, L. and Dagnelie, J., Bull. d'Histol. Appl., 1935, **12,** 85–91). To stain *lipoid granules in leucocytes.* Dry blood smear and fix in methyl alcohol, 30 sec. Stain in a jar with sat. Sudan black B in 70% alcohol, 30 min. Rinse in water and wash 1 min. in 70% alcohol to remove deposit. Counterstain with sat. alcoholic eosin in 70% alcohol, 30 sec. Wash and stain in sat. aq. methylene blue 3 min. Rinse, blot dry and examine with oil immersion. Lipoid granules, deep black; nuclei, blue; and erythrocytes, red. (Sheehan, H. L., J. Path. & Bact., 1939, **49,** 580–581).

**Sudan Black $B_1$** as a bacterial fat stain. Sat. sol. of Sudan black B (Nat. Aniline and Chemical Co.) in 70% alcohol, or in ethylene glycol stains fat bodies in bacteria deep blue black (Hartman, T. L., Stain Techn., 1940, **15**, 23–28).

**Sudan G**, see **Sudan III.**

**Sudan Hydrotropes.** Sudan stains are relatively insoluble in water. They can be changed to hydrotropes (Neuberg) which are water soluble. The hydrotropes of red lipid stains are of a blue color which changes to red when the dye passes into a lipid or a lipid solvent. This is the basis of a useful technique for lipids (Hadjioloff, A., Bull. d'Hist. Appl., 1938, **15**, 37–41).

**Sudan R** (CI, 113)—brilliant fat scarlet B, oil vermillion—A weakly acid mono-azo dye.

**Sudan Red**, see **Magdala Red.**

**Sulfmethemoglobin**, a greenish compound of methemoglobin and sulphur often encountered in abdominal walls of cadavers, but it may be present in blood where it can be diagnosed by spectroscopic examination (Mallory, p. 135).

**Sulfonphthaleins.** These are compounds of phthalic anhydride and ortho-sulfobenzoic acid. They are most valuable indicators. Examples: brom chlor phenol blue, brom cresol green, brom cresol purple, brom phenol blue, brom phenol red, brom thymol blue, chlor cresol green, chlor phenol red, cresol red, metacresol purple, phenol red, thymol blue.

**Sulphonal Poisoning.** Effect on liver cell mitochondria (Grynfeltt, E., and Lafont, R., C. rend. Soc. de Biol., 1921, **85**, 406–408).

**Sulphur.** In inorganic form sulphur is not found in living things except in the thiobacteria. Histochemically one has to consider sulphates and masked sulphur. Macallum has devised a method for sulphates but Lison (p. 121) says that it only gives very rough localization in tissues because the salt is diffusible. For organic, masked sulphur see **Sulfmethemoglobin, Glutathione, Radiosulphur.**

**Sultan Red 4B**, see **Benzopurpurin 4B.**

**Supravital Staining.** By this is meant staining upon the living state. In other words stains are applied to cells removed from a living animal, or to cells within a recently killed animal. Thus blood cells are removed from the body and, while still living, are stained supravitally or the stains are applied to still living cells of, say, the stomach within the body of a recently killed animal by vascular injection. The essential point is that the stains act upon living cells but the cells do not go on living, neither does an animal injected intravascularly with a supravital stain. Janus green is our most useful supravital stain. Cells supravitally stained by it die and when it is injected in sufficient quantity into a living animal, the animal dies likewise for it is toxic. *Vital stains*, on the contrary, do not kill cells and can be safely injected into living animals since they are nontoxic in the concentrations necessary to obtain the desired results. This kind of staining used to be called *intravital* in contrast to *supravital*. *See* **Vital Stains.**

Supravital stains have been known for a long time but their introduction as essential means of investigation is due primarily to Professor R. R. Bensley of the University of Chicago (Am. J. Anat., 1911, **12**, 297–388). He showed their usefulness in demonstrating specifically by vascular injection the different epithelial components of the pancreas and he called attention to the fact that to stain mitochondria specifically it is essential to use janus green having the composition of *diethyl*safranin-azodimethylanilin, that the dimethyl compound will not work. The supravital staining of blood cells began with the demonstration by Cowdry at Hopkins (Internat. Monatschr. f. Anat. u. Physiol., 1914, **31**, 267–286), that this particular janus green B as used in Bensley's laboratory stains the mitochondria in human white blood cells specifically. The method was later further developed by Sabin and her associates. Details of techniques are given under janus green, neutral red, brilliant cresyl blue, pyronin, methylene blue, naphthol blue and cyanamin. Useful table giving reactions of types of blood cells (Gall, E. A., J. Lab. & Clin. Med., 1934–35, **20**, 1276–1293).

**Surface Measurements.** To determine the surface area of structures of microscopic size involves many techniques some of which are rather complicated. The following references are given to methods and results in a wide variety of instances. Perhaps the particular surface to be measured will be sufficiently similar to one of these to justify employment of the same technique or a modification of it.

Endothelium of vascular capillaries—6300 sq. meters—Krogh, A., Anatomy and Physiology of Capillaries, Yale Press, 1929, 422 pp.

Erythrocytes combined—3500 sq. meters—Evans, C. L., Recent Advances in Physiology. Philadelphia: Blakiston, 1926, 383 pp.

Filtration surface of both kidneys combined—1.56 sq. meters—Vimtrup,

B. J., Am. J. Anat., 1928, **41**, 132–151. See also recent measurements for albino rat by Kirkman, H. and Stowell, R. E., Anat. Rec., 1942, **82**, 373–389.

Gastric glands secreting surface—2.7 sq. meters—Scott, G. H. (personal communication), see Cowdry's Histology (p. 282).

Lacteal surface in small intestine—5 sq. meters—Policard, A., Précis d'Histologie Physiologique. Collection Testut, Paris: G. Doin, 923 pp., after Potter.

Large intestinal crypts—4.2 meters—Policard, *ibid.*

Mitochondrial, zymogenic and nuclear surfaces in pancreatic acinous cells of guinea pig—duNouy, P. L. and Cowdry, E. V., Anat. Rec., 1927, **34**, 313–329.

Respiratory surface plus nonrespiratory epithelial surface of airways of lungs—70 sq. meters—Wilson, H. G., Am. J. Anat., 1922, **30**, 267–295.

**Surface Tension.** About this much has been discovered and written (Reviews: Harvey, E. N., and Danielli, J. F., Biol. Rev., 1938, **13**, 319–341 and Danielli, J. F. in Bourne, pp. 69–98). Before measurements can be made on cells it is obviously necessary to isolate them and this entails risk of injury which is much greater in the case of mammalian cells than of the sea urchin eggs usually employed. The following techniques are given as examples:

1. By centrifuging marine eggs elongation can be produced and, when the length exceeds a certain ratio of diameter, the egg divides. Knowing the minimum force required, the difference in density between the 2 halves and the circumference of the cylinder, it is apparently possible to calculate the tension at the surface (Harvey, E. N., J. Franklin Inst., 1932, **214**, 1–23).

2. By compressing sea urchin eggs by a minute gold beam the internal pressure can be calculated and from this the surface tension (Cole, K. S., J. Cell & Comp. Physiol., 1932, **1**, 1–9).

3. By stretching a cell between the two needles of a microdissection apparatus the force required to secure a given degree of elongation can be determined and thence the surface tension (Norris, C. H., J. Cell & Comp. Physiol., 1939, **14**, 117–133).

4. Surface tension is probably to some extent at least conditioned by the elasticity of the superficial plasma gel layer which brings in the methods and observations of Lewis, W. H., Arch. f. exp. Zellf., 1939, 28, 1–7; Am. J. Cancer, 1939, **35**, 408–415 who refers to previous work along this line.

**Swiss Blue,** see **Methylene Blue.**

**Susa** fixative of Heidenhain. Corrosive sublimate, 4.5 gm.; common salt, 0.5 gm.; aq. dest., 80 cc.; formalin, 20 cc.; and trichloracetic acid, 4 cc. Fix about 12 hrs., wash in 95% alcohol. It has been modified by several people. See **Buzaglo.**

**Swiss Blue,** see **Methylene Blue.**

**Synapses,** see methods employed by Bartelmez, G. W. and Hoerr, N. L., J. Comp. Neurol., 1933, **57**, 401–428.

**Synovial Fluid** of normal knee joint. Method of examination and results (Coggeshall, H. C., Warren, C. F. and Bauer, W., Anat. Rec., 1940, **77**, 129–144).

**Taenia Echinococcus,** a parasite of dogs which produces hydatic cysts in human liver and other tissues. The laminated cyst wall is typical and the heads have double circle of hooks and 4 suckers.

**Taenia Saginata.** In examination of fresh **Feces** identify by head with 4 suckers but without hooks.

**Taenia Solium.** Look in **Feces** for head with 4 suckers and a circle of small hooks best seen in fresh mounts. The genital system opens at the side and the uterus is only slightly branched.

**Tapeworm Proglottids.** Orient pieces 4–5 cm. long containing gravid proglottids between glass slides held together by elastic bands. Fix in Bouin's fluid (sat. aq. picric acid, 7 parts; glacial acetic acid, 20 parts; and formalin, 10 parts 10–12 hrs. Wash in running water 2–3 min. Flood with 10% aq. NaOH (outlines of uterus become visible deep orange). Rinse in tap water. Flood with 5% HCl 1–2 min. Tap water 10–15 min. Dehydrate in alcohol, clear in xylol and mount in balsam (Dammin, G. J., J. Lab. & Clin. Med., 1937–38, **23**, 192–194). See **Parasites.**

**Tarsal Glands.** Whole mounts can be made by the method described for **Sebaceous Glands.** They are also known as Meibomian glands.

**Taste Buds.** To demonstrate, choose circumvallate papillae, fix in **Bouin's Fluid** and stain with **Hematoxylin and Eosin.** See Arey, L. B. et al., Anat. Rec., 1935–36, **64**, 9–25.

**Teeth.** The most comprehensive statement of microscopical technique is contained in A. W. Wellings' "Practical Microscopy of the Teeth and Associated Parts." London: John Bale Sons & Curnow Ltd., 1938, 281 pp. A chapter by Churchill and Appleton in McClung's Technique is also useful. Teeth can be studied from so many different angles that to outline the techniques in a few words is extraordinarily difficult. Their composition of (1) enamel, the hardest tissue in the whole body, with (2) dentin

which is highly mineralized and contains the processes of cells but not their nucleated bodies plus (3) richly cellular pulp confers numerous obstacles. The wise histologist or pathologist will save valuable time by at once seeking advice from experts in some dental research laboratory. They possess experience and instruments for grinding and sawing both of which he lacks. Teeth of adults can be prepared for examination in 2 principal ways:

1. *Without decalcification.* Churchill and Appleton (McClung, p. 253) recommend, in place of the usual grinding method, a cutting technique used by Johnston at Yale. After extraction fix the tooth immediately in formalin. Then dry and fix to wooden block by modelling compound. Sections are then made by the cutting wheels of a power lathe. If necessary they are polished on a Belgian stone, dehydrated in alcohol, cleared in xylol and mounted in balsam.

When one wishes to include the soft as well as the hard parts Chase's technique of *petrifaction* is advised by them. Fix as desired (say 10% formalin) and wash as required. Transfer to aq. gum arabic or dextrin of syrupy consistency. Freeze on freezing microtome and cut slices with very fine saw (jeweler's). Remove gum arabic by washing in water and stain with carmine or hematoxylin. Dehydrate through alcohols to 95%, ½ to several hours each depending on size of slice. Acetone ½ hr. or more. Cover with thin celloidin in a container to depth twice or more thickness of slice. Leave container top open very slightly permitting evaporation until celloidin will scarcely flow when container is steeply tilted. Transfer with considerable celloidin to container of heavy lead foil and further evaporate until completely hardened. Grind and polish both sides of slice in presence of water. Remove celloidin with acetone and acetone with xylol. Mount in balsam. Sections obtained by this and the Johnston technique can be examined by direct illumination, in the dark field, in ultraviolet light (Walkhoff, O., Dental Cosmos, 1923, 65, 160–176), in polarized light (Andresen, V. The Physiological and Artificial Mineralization of Enamel. Oslo. Dancke, 1926) and by x-ray for which many references are given (McClung, 381–385).

2. *With decalcification.* In the *paraffin technique*, advised by Churchill and Appleton, clip ends of roots of a freshly extracted tooth or drill hole. Fix in 4% formalin. Dry with towel and seal openings to pulp with celloidin. Quickly dry. Decalcify in 10% hydrochloric acid C.P. 10 days or more testing with needle. Running water, 24 hrs. 95% alc., 24 hrs. Abs. alc., 5 hrs. Chloroform, 1 hr. Equal parts chloroform and 45°C. paraffin in glass stoppered bottle on top of oven (oven 58°C.) over night. ½ hr. each in following paraffins (1) 42–46°C., (2) 52–56°C. and (3) 58–60°C. within oven. Imbed in a mixture of 235 cc. 52–56°C. paraffin and 15 cc. beeswax. See **Paraffin Sections.**

In the *celloidin technique* (Churchill and Appleton) cut off apex of tooth or drill a hole to pulp through crown. Fix in 4% formalin, buffered to counteract acid, 45 hrs. for single teeth. (Wash in water) change to 80% alc. 95% alc. 2 weeks +. depending on size. Abs. alc. 2 weeks +, abs. alc. (exposed to anhydrous copper sulphate, see **Alcohol**) 2 weeks +. Equal parts abs. and ether, 2 weeks +. Then 1 month or more in ½, 1, 2, 5, 7, 10, 12% celloidin (parlodion). Orient and imbed in 12% in stender dish. Make depth of celloidin twice height of tissue. Place lid of stender dish on tightly. Allow bubbles to rise 24 hrs. If bubbles still present move tissue gently so they can escape. Put piece of paper between lid and dish, 24 hrs. +. Evaporate to consistency hard rubber, 7 days +. 80% alc. 48 hrs. or until beginning decalcification. Trim block leaving sufficient celloidin about tissue to facilitate cutting. 10% acetic or hydrochloric acid in 70% alc. changing daily 3 weeks + until needle penetrates easily. When spaces appear in the celloidin drill holes to reach them. Wash 24 hrs. in running water; then same time in weak sol. sodium bicarbonate. Wash 24 hrs. + in water. 50, 70 and 80% alc. each 24 hrs. +. 95% and abs. alc., ½ hr. each. Alc. ether, 0.5% and 12% celloidin 5–20 min. each. Harden in chloroform, 24 hrs. Leave in 80% until sections are made, see **Celloidin Sections.**

For small and developing teeth a wider variety of methods is possible see **Teeth Developing.** To classify examples of all the methods available for old and young teeth and associated structures in a manner expected by the reader is not feasible. In general however there are methods that involve whole teeth which come under **Teeth (Blood Vessels, Innervation, Lymphatics)** and their response to **Alizarin Red** staining and exposure to **Radioactive Phosphorus.** Some techniques are also provided under **Teeth and Jaws** and parts of teeth: **Enamel, Dentin,** and **Pulp.**

**Teeth, Blood Vessels** (Boling, L. R., Anat. Rec., 1942, 82, 25–32). Two suspensions

are recommended: (1) cinnabar, 120 gms.; gum arabic, 40 gms.; water, 160 cc. (2) cinnabar (red mercuric sulphide), 80 gms.; corn starch, 40 gms.; 10% formalin in physiological saline, 125 cc. Grind up the mixtures slowly in a glass ball mill for 2 or 3 days, strain through gauze, and use immediately. Anesthetize a cat or dog with sodium pentobarbital. Expose and ligate both common carotid arteries. Perfuse the head with physiological saline through a glass cannula inserted in one carotid. Incise the carotid of the opposite side distal to the ligature and allow it to bleed until clear saline appears when it should be clamped. Open the jugular veins and allow them to drain. As soon as all blood has been washed from the vessels of the head direct the suspension through the same cannula by means of a two way stop cock. Maintain a pressure of 120 mm. of mercury by air pressure. Aid penetration by gentle rhythmic pressure on a hand bulb inserted in the conducting system. When injection of the mass is begun remove the clamp momentarily from the opposite carotid to allow free flow of the mass in all large arteries. This promotes good injections of both right and left sides from the single cannula. After completion of the injection remove the head and place in strong formalin over night, then cut away the soft tissue from the jaws and place the jaws in10% formalin in saline solution for several days, wash, and decalcify in 5% nitric acid. After decalcification dehydrate thoroughly in graded series of alcohol and clear in two changes of methyl salicylate. Dissect away any bone interfering with observation of teeth. This is best done with a dental engine and round bur while the specimen is immersed in clearing fluid. Moisture or heat will cause clouding of the specimen and must be avoided. In addition to the desirable color of cinnabar, is the radiopacity of these injections; the course of all macroscopically visible vessels may be followed in roentgenograms before decalcification. The method also works well on soft tissues. The first mass will pass through all capillaries in a tooth and fill both arteries and veins. Better demonstration of arteries is obtained with the second which has not been found to pass through capillaries. The use of formalin seems to aid in the retention of the mass in the blood vessels and to prevent the formation of gas bubbles in the pulp cavity during decalcification.

**Teeth, Decalcification**: Details from Dr. L. R. Boling, Washington University (School of Dentistry).

Decalcification of teeth for the preparation of histological sections presents several problems not encountered with other tissues especially if the surrounding bone and soft tissues are also preserved. The great difference in salt content and organic matrix of enamel, dentin, cementum, bone and soft tissues makes difficult the preservation of one while the others are being decalcified.

Enamel, except in the most immature portions of developing teeth, is entirely destroyed by ordinary decalcification methods. The organic portion of adult enamel may be observed by the slow decalcification of thin ground sections under a cover slip (Chase, S. W., Anat. Rec. **36**, 239–258, 1927). The acid, one per cent nitric, hydrochloric or sulphuric, or five per cent chromic, acetic or citric, is run under a propped cover slip over the section. Action may be stopped at any point by substituting water for acid and the remaining material stained and mounted as desired without disturbance. Boedeker's method of "celloidin-decalcifying" is also said to give good results (Fundamentals of Dental Histology and Embryology, New York, The MacMillan Co., 1926, p. 223) and allows sectioning of the organic remainder in any plane. See **Enamel.**

For the examination of sections of whole teeth without enamel or for teeth in relation to the bone of the jaws five per cent nitric acid in water has been found by most investigators to give consistent results. Hydrochloric acid may be used but causes too much swelling. Organic acids such as formic, acetic, picric, etc., can be used as for bone but ordinarily give no better results than nitric. For delicate objects one to five per cent nitric acid in 70 per cent alcohol may prove superior. The highly recommended phloroglucin nitric acid (phloroglucin 1 gm., nitric acid 5 cc. or more, 70 per cent alcohol 100 cc.) seems to have no advantage when used on teeth and if the stronger acid solutions are used it can be destructive because the outer tissues are over decalcified before the deeper structures are prepared.

Celloidin imbedding before decalcification helps preserve tissue relationships (See **Teeth,** celloidin technique). Arnim has perfected a technique of double imbedding for rat jaws and teeth which, though tedious, yields beautiful results (Anat. Rec. **62**, pp. 321–330, 1935). This method has been modified by Burket for larger teeth (McClung p. 366).

Tooth buds may be decalcified after paraffin imbedding by the following method given by Dr. L. R. Boling in a personal communication. Carefully remove from the tooth bud all surrounding bone. Fix, dehydrate, clear and imbed in paraffin in the usual way. Shave away paraffin and soft tissue from one surface of the specimen so that enamel is exposed. Immerse block in 5 per cent aq. nitric acid until decalcification is complete. Place in 5 per cent aq. sodium sulphate for a few hours. Wash over night in running water and reimbed, handling the tissue as gently as possible in order not to disturb relationship of hard and soft tissues. This method permits demonstration of Golgi apparatus and mitochondria in ameloblasts and odontoblasts *in situ*. It works best with teeth of small animals easily penetrated by fixative. The paraffin protects the soft tissues but does not interfere with action of acid on enamel and dentin. (See also **Teeth, Developing**).

Successful preparation of decalcified tooth sections depends as much or more on the care of the tissues before and after decalcification than on the actual process. Good fixation of the pulp tissue is difficult but essential to prevent shrinkage. Ten per cent formalin in physiological salt sclution may be used for several days or weeks without injury to the soft tissue and allow thorough penetration. Better results are obtained in a short time if the fixative can be perfused through the blood

Cutting of holes through the dentin to the pulp or the amputation of the tips of teeth is often resorted to in order to get better penetration but these methods are apt to disturb the position of the pulp and should be avoided if possible. After decalcification the teeth should be carefully handled and the dehydration process should be slow to prevent separation of tissues of different densities.

Over decalcification should be carefully avoided because it will partially destroy the dentin matrix, cause separation of tissues of differing consistency and disturb staining reactions. Testing for completion of decalcification by probing with needles or bending and squeezing in the fingers should be avoided at all costs if tissue relationships are desired. The progress of decalcification can be followed radiographically but the end point can not be accurately determined. The best method of testing is that described by Arnim (loc. cit.). Five cc. of the acid used in decalcification is placed in a clean test tube and neutralized with ammonium hydroxide, and .1 cc. of a saturated solution of ammonium oxalate added. If no precipitate forms additional .1 cc. portions of oxalate are added at 15 minute intervals until .4 cc. have been added. If a precipitate is formed the tissue is placed in fresh acid and retested in 24 hours. Formation of no precipitate with .4 cc. oxalate solution after 24 hours in fresh acid is indicative of complete decalcification.

When tissues are found to be not sufficiently decalcified after imbedding the process can be completed by immersing the celloidin block in acid 70 per cent alcohol or floating the paraffin block, cut surface down, on acid if the dentin is exposed.

**Teeth, Developing.** 1. *Tooth germs.* Glasstone (S., J. Anat., 1935–36, **70** 260–266) has described a method for the excision of tooth germs from 18–21 day rat embryos and their **Cultivation** in fowl plasma and embryo extract. The technique of **Transplantation** of tooth germs of young pups into the abdominal wall has been reported by C. H. Huggins et al. (J. Med., 1934, **60**, 199). Bevelander, G., Anat. Rec., 1941, **31**, 79–97 obtained fine preparations of Korff's fibers in pig's tooth beginning with 110 mm. stage by fixation in **Formalin-Zenker** and silver impregnation by **Foot's Method.**

2. *Young teeth.* Beams, H. W. and King, R. L., Anat. Rec., 1933, **57**, 29–40 fixed the developing molar teeth of white rats 1–5 days old in a variety of fluids. They employed the **Nassonov** technique for the Golgi apparatus and **Regaud's** for mitochondria without any special provision for decalcification. In some cases *Boling's Decalcification* (**Teeth, Decalcification**) method after paraffin imbedding may prove useful. Dr. Boling states in a personal communication that a modification of Bouin's picro-formol fixative may be used for fixing and decalcifying very young tooth buds or teeth and jaws of rats. A mixture of 75 parts saturated aqueous solution of picric acid, 25 parts formalin and 10 to 20 parts glacial acetic acid will decalcify a mature rat jaw and teeth in less than a week. Ordinary Bouin's picro-formol is sufficiently acid to decalcify very young tooth buds in a few days. After decalcification the tissues are handled in the same manner as soft tissues after Bouin fixation except that a longer period is allowed for removal of picric acid. This procedure allows better than average staining of decalcified tissues. Nuclear structure is especially well preserved and little

separation of hard and soft tissues is found. The method of microincineration has been adjusted to developing teeth by Hampp, E. G., Anat. Rec., 1940, 77, 273–286.

**Teeth, Innervation.** Methods described under **Nerve Endings** require considerable adaptation before they can be employed for the teeth. For obvious reasons methylene blue is particularly difficult to use. From a great many techniques 2 are selected.

1. Van der Sprenkel, H. B., J. Anat., 1935–36, **70**, 233–241. Grind dentinal wall of normal human canine tooth down to a thickness of 300–500 microns leaving the cavity closed and the pulp untouched. Saw remainder of tooth into rings (not decalcified). From them cut on freezing microtome cross sections about 40 $\mu$ thick and impregnate according to the Gros method. Van der Sprenkel does not give a reference to this method. Perhaps the Gros method, as given by Lee (p. 494) will serve. Treat frozen sections with pyridine. Wash with aq. dest. to remove odor of pyridine. 20% aq. silver nitrate, in dark, 1 hr. Transfer without washing to 20% formalin neutralized with magnesium carbonate. Change twice until no more white ppt. is formed. Reduce under microscope in following solution: Add ammonia to 15 cc. 20% silver nitrate until ppt. formed just disappears. Then add 1 drop ammonia per each cc. silver nitrate solution. After this 20% aq. ammonia 1 min. or more. 1% acetic acid, same. Tone in 0.2% aq. gold chloride treat with sodium hyposulphite, wash, dehydrate, clear and mount. Counterstain with **Van Gieson** or toluidin blue, if desired before dehydration. See Van der Sprenkel's illustrations.

2. Christensen, K., J. Dent. Res., 1940, **19**, 227-242 was concerned primarily with determination of the source of the large proportion of unmyelinated and small myelinated fibers in the pulp. His technique is a combination of dissection and the making of histological preparations of cats. First inject arteries with a yellow corn starch mass (composition not specified) and harden tissues in formalin. Expose cervical sympathetic, common carotid and its chief branches, mandibular canal and floor of orbit. Wash dissected areas with aq. dest., and brown nerves with dilute aq. silver nitrate so that they can be easily followed along the walls of the yellow colored vessels. To trace their final path to lower teeth serial sections of inferior alveolar nerve and artery are required and to upper teeth similar ones of internal maxillary plexus and superior alveolar nerves. Wrap canine teeth in cotton, carefully crack with vise and remove pulps. Slightly stretch each pulp along surface of short glass tube attaching the ends to the tube by silk threads to prevent tortuosity of nerve fibers in the final preparations made by the **Bodian-Method.** Examine the cervical sympathetic ganglia by techniques for **Nissl Bodies** as well as for nerve fibers before and after degeneration resulting from experimental destruction of dental pulp.

**Teeth and Jaws.** Sections through (Willman, M., J. Dental Res., 1937, **16**, 183–190). Fix in 10% formalin, 10–30 days. Transfer to 95% alcohol for same time. After decalcification in 5% aq. nitric acid, change to 5% aq. sodium sulphate for 24 hrs., then wash in running water 24 hrs. Dehydrate through ascending alcohols to 95%, then 2 changes of absolute, 6%, 12% and 25% celloidin solution, 7 days each. Cut sections with heavy, sledge type of microtome. Remove celloidin from sections with alcohol-ether and pass down to aq. dest. Stain with Harris' hematoxylin and acid alcohol eosin. Mount in dammar. Control decalcification either by testing a second tooth with a needle or by polariscope. See **Dental Enamel.**

**Teeth, Lymphatics.** Obviously the work of Fish, E. W., Proc. Roy. Soc. Med., 1926–27, **20** (3), 225–236; Bödecker, C. F., and Lefkowitz, W., J. Dent. Res., 1937, **16**, 463–475 and others relating to the "lymph supply" of dentin and enamel does not refer to lymph but to tissue fluid for the spaces are not lined with lymphatic endothelium. For tissue fluid in these situations see Cowdry, E. V. Problems of Ageing. Baltimore: Williams & Wilkins, 1942, p. 593. An excellent account of techniques designed for investigation of the lymphatic system of teeth and jaws is provided by MacGregor, A., Proc. Roy. Soc. Med., 1935–36, **29** (2), 1237–1272. His favorite injection masses were strong solutions of basic lead acetate and acid suspensions of carmine. Before killing and injecting the animals (cats, dogs, guinea pigs and monkeys) he caused them to inhale large doses of amyl nitrite with the idea of dilating the peripheral blood vessels.

**Teichmann,** see **Hemin Crystal Test.**

**Tellyesniczky's** fixative. 5 parts of formol, 100 of 70% alcohol and 5 of acetic acid.

**Tendons.** These are dense bands of collagenic fibers interspersed by a few flattened fibroblasts (lamellar cells). Fixatives penetrate the larger ones poorly. **Zenker's Fluid** and **Hematoxy-**

**lin** and **Eosin** are fairly satisfac'ory. For mechanical factors in structure see Carey, E. J., Am. J. Anat., 1936, **59**, 89–122; Anat. Rec., 1936, **64**, 327-3 1.

**Terpineol** (or terpinol), a mixture of substances of composition $C_{10}H_{16}$ and $C_{10}H_{18}O$ formed by action of dil. HCl on terpin hydrate. Used as a clearing agent. Can clear tissues from 90%, even from 80% alc. A good mixture is 4 parts terpineol + 1 part xylol.

**Tertiary Butyl Alcohol** (trimethyl carbinol). Has been recommended as a substitute for ethyl alchol and clearing agents like xylol in the paraffin technique because it mixes easily both with water and paraffin. It causes but little shrinkage and hardening of tissue. One method (Stowell, R. E., Science, 1942, **96**, 165–166) is partly to substitute for ethyl alcohol by passing through the following series of mixtures: (1) Aq. dest., 50 cc.; 95% ethyl, 40 cc.; butyl, 10 cc.; 1–2 hrs. (2) Aq. dest., 30 cc.; 95% ethyl, 50 cc.; butyl, 20 cc., 2 hrs. to several days. (3) Aq. dest., 15 cc.; 95% ethyl, 50 cc.; butyl, 35 cc.; 1–2 hrs. (4) 95% ethyl, 45 cc.; butyl, 55 cc.; 1–2 hrs. (5) Butyl, 75 cc.; abs. ethyl, 25 cc.; 1–3 hrs. (6) Pure butyl, 3 changes 4 hrs. to overnight. (7) Equal parts pure butyl and paraffin oil, 1–2 hrs. Infiltrate in paraffin. Another method (Stowell, R. E., J. Tech. Methods, 1942, **22**, 71–74) is to entirely substitute 50%, 70%, 85% and pure butyl alcohol for the corresponding ethyl alcohols. Stowell provides useful suggestions as to the details of paraffin imbedding. Tertiary butyl alcohol has been recommended for dehydrating material stained with methylene blue and other dyes readily extracted during ethyl alcohol dehydration (Levine, N. D., Stain Techn., 1939, **14**, 29–30). It may be used as a substitute for ethyl alcohol in the acid fast and Gram stains for bacteria (Beamer, P. R. and Stowell, R. E., in press). Do not confuse with *n* Butyl alcohol.

**Testis.** Methods described elsewhere for the **Connective System, Blood Vessels, Nerve Fibers** and so on are available. Technique for isolation of seminiferous tubules is given under **Maceration.** See also **Chromosomes.** Wagner, K., Biologia Generalis, 1925, **1**, 22–51 has employed a method of vital staining with trypan blue which he claims differentiates between interstitial cells and histiocytes or macrophages. Duesberg, J., Biol. Bull., 1918, **35**, 175–198, using the **Benda Method,** obtained preparations of opossums which he thought indicated discharge of material from the interstitial cells into the blood stream. Wagner (*loc. cit.*) has observed somewhat similar phenomena in other animals, but there has been no satisfactory follow up. For detailed information about interstitial cells see Rasmussen, A. T., Cowdry's Special Cytology, 1932, **3**, 1674–1725.

**Tetrachrome Blood Stain,** see **MacNeal's.**

**Tetralin** is tetrahydronaphthalene used as a clearing agent after **Diaphanol.**

**Thallium.** Barbaglia's Method. Fix in 95% alcohol iodized. This precipitates thallium in the form of insoluble crystals of thallium iodide recognizable by their yellow color (Lison, p. 66).

**Thiamine.** Blaschko and Jacobson (H. and W. in Bourne's Cytology, 1942, p. 196) refer to the work of Ellinger and Koschara in the observation under the fluorescence microscope of green fluorescence due to flavin and that on alkalinization this is replaced by a bluish fluorescence which is known to be occasioned by the presence of thiamine, itself identical with vitamin B, or aneurin.

**Thiazin Dyes.** A very useful group of dyes for the histologist. The two benzene rings are joined by =N– and –S–. Examples: azure A, B and C, methylene azure, methylene blue, methylene green, methylene violet, new methylene blue N, thionin, toluidin blue O.

**Thiazine Red R** (CI, 225)—chlorazol pink Y, rosophenine 10B—An acid mono-azo dye employed especially as counterstain for iron hematoxylin.

**Thiazole Dyes** contain thiazole ring with indamine as chromatophore. Geranine G, primalin, thioflavine S, and titan yellow. All of these dyes appear to be useful in fluorescence microscopy. Pick, J., Zeit. f. wis. Mikr., 1935, **51**, 338–351 refers to three of them.

**Thiazole Yellow,** see **Titan Yellow.**

**Thioflavine S** (CI, 816). An acid thiazole dye used in fluorescence microscopy.

**Thionin** (CI, 920)—Lauth's violet—Commission Certified. An extremely useful basic thiazin dye. See **Tissue Basophiles, King's Carbol Thionin,** etc.

**Thorium Dioxide** is occasionally employed as a vital stain for reticulo-endothelium. Angermann, M. and Oberhof, K., Zeit. f. Ges. Exp. Med., 1934, **94**, 121–126 give directions for its administration to rabbits and for determination of its distribution chemically, radiologically and histologically. (Thorotrast)

**Thyme Oil** N.F. VI. Sometimes misnamed oil of origanum. Contains thymol, carvacrol, cymene, pinene, linalool and bornyl acetate. It is said to be useful for clearing celloidin sections.

**Thymonucleic Acid** (*Feulgen or nucleal reaction for*). Pass paraffin sections, fixed in equal parts sat. aq. corrosive sublimate and absolute alcohol, through xylol

and alcohols to water. Place in a staining jar containing normal HCl (82.5 cc. HCl, sp. gr. 1.17–1.185 per liter of water) at room temperature for 1 min. Transfer to normal HCl, at 60°C. and there hydrolyze for 4 min. Treat with the fuchsin sulphurous acid reagent in a staining jar for ½–1 hr. (This reagent is: One gram of basic fuchsin is dissolved in 100 cc. of distilled water with the aid of a little heat. The solution is filtered while still warm and 20 cc. of normal HCl is added to the filtrate. The resulting fluid is then cooled and 1 gm. dry sodium bisulfite ($NaHSO_3$) is added. Then, after standing for about 24 hrs., the reagent is ready for use and should have a pale straw color.) Pass through a series of 3 jars, each containing a solution made by adding 10 cc. of a molecular solution of sodium bisulfite (i.e., 104 grams per liter) to 200 cc. of tap-water, allowing 1½ min. in each and agitating frequently. Wash in tap water for 5 min., dehydrate, clear and mount in balsam. Thymonucleic acid is colored purple or violet and color holds (Cowdry, E. V. Science, 1928, **68**, 40–41). Collected references (Milovidov, P., Protoplasma, 1938, **31** (2), 246–266); technique for plant tissues (Whitaker, T. W., Stain Techn., 1939, **14**, 13–16). See Bauer-Feulgen stain for **Glycogen.**

**Thyroid.** For routine purposes Zenker fixation and hematoxylin and eosin staining of paraffin sections is suggested. If one is interested in the *colloid*, its appearance after various fixations, its shrinkage patterns and the significance of its acidophilic and basophilic staining are described by Bucher, D., Zeit. f. Zellf. u. Mikr. Anat., 1938, **28**, 359–381. The effect on colloid of different agents for dehydration and clearing is described by Ralph, P., Stain Techn., 1938, **13**, 9–15. A method for determination of the volume of colloid is given by Stein, H. B., Am. J. Anat., 1940, **66**, 197–211.

The shape of thyroid *follicles* can be distinguished but imperfectly in sections unless reconstructions are made from serial sections. For an excellent method of viewing entire, isolated follicles see **Maceration.** The localization of unsuspected masses of follicles, not present in the gland, in the neck tissues of experimental animals can be accomplished by supravital staining with **Naphthol Blue.**

Many methods are available for the detailed examination of the secretory *epithelial cells* not requiring their special adjustment to the thyroid gland. See **Mitochondria, Microchemical** methods, etc. The **Brazilin-Wasserblau** technique is recommended for intracellular *secretion antecedents*. If the Golgi apparatus is to be investigated consult Welch, C. S. and Broders, A., Arch. Path., 1940, **29**, 759–772. A fine beginning has been made in the direct study of vacuoles within the follicles in living mice by transillumination after the fashion of Knisely (Williams, R. G., Anat. Rec., 1941, **79**, 263–270). Minute instructions for demonstration of blood vessels and lymphatics and results which are to be expected are given by Rienhoff, W. F., Arch. Surg., 1931, **23**, 783–804. For fluorescence see Grafflin, A. L., J. Morph. and Physiol., 1940, **67**, 455–470.

**Ticks.** The following method for softening and sectioning is an adaptation by Miss Slifer of the Slifer-King technique for grasshopper eggs (Slifer, E. H., and King., R. L., Science, 1933, **78**, 366–367). Drop animal into dish of **Carnoy-Lebrun.** After 5 min. place under binocular and puncture with a glass needle. Allow fixative to act for at least 20 min. longer. (Variations in the size of the puncture and in the length of time for fixation should be tried.) Transfer to 70% alcohol colored a light yellow with iodine over night. If alcohol is colorless next morning let stand a few hours longer. Repeat if necessary. At this point (or somewhat earlier) it is well to make a larger incision in the animal with a scalpel. The viscera should now be well-hardened and should not ooze out through the hole. 70% alcohol, several hrs. 70% alcohol containing 4% phenol, 2 or 3 days. 95% alcohol 2 hrs. Anilin oil, several hrs. Chloroform (2 changes of 5 min. each). Paraffin about an hour. Imbed and block. Trim block away so that viscera are just exposed, at the point where sectioning is to begin. Place block in water containing 4% phenol. *Be sure that the cut surface is under water and examine occasionally to see that air bubbles do not form on it.* After 3 days a swelling of the tissues should be noticeable so that they protrude a little beyond the cut surface of the paraffin. If this has not occurred, cut away a little more and soak several days longer. Trim block, place on microtome and section 5–7 microns. *Work rapidly once you have begun.* A slight delay between sections will allow the cut surface to dry. If, for any reason, it is necessary to stop wet a scrap of paper and stick it to the cut surface. In case of difficulty in making sections stick to slides try Haupt's gelatine fixative (Stain Techn., 1930, **5**, 97–98). After the sections have been spread, arranged on the slide and

excess liquid drained off, place a piece of damp (not wet) filter paper (or good quality paper toweling) on the table and press the slide, *section side down*, very firmly against it. Dry. See **Chitin** for other methods of softening.

**Tigroid Bodies** (G. *tigris*, tiger and *eidos*, appearance). A term applied to Nissl bodies since they sometimes look streaked and spotted like a tiger. See **Nissl Bodies.**

**Tissue Basophiles** (tissue mast cells). Some think that these cells are emigrated **Basophile Leucocytes** and others that they are of extravascular origin. They can easily be studied in fresh spreads of **Loose Connective Tissue** or omentum. Their granules are readily colored supravitally with brilliant cresyl blue, methylene blue and other stains. Tissue basophiles disintegrate quickly. Maximow, A., Arch. f. mikr. Anat., 1913, **83** (1), 247–289 gives the following metachromatic stain for mast cells. Sections of abs. alc. fixed tissues are stained 24–48 hrs. in sat. thionin in 50% alc. Staining can be reduced to 20 min. by adding 4 drops 3% $Na_2CO_3$ to 20 cc. thionin sol. and filtering before use. Maximow gives technique for smears and spreads fixed in formalin Zenker. See his beautiful colored plates. See **Toluidine Blue Phloxinate.**

Holmgren and Wilander (H. and O., Ztschr. f. mikr. Anat. Forsch., 1937, **42**, 242–278) recommend fixation in 10% aq. basic lead acetate and staining with 1% alc. Toluidin blue. They show that fixation in formalin-alcohol gives very inferior results. In their opinion the metachromatic substance colored is identical with **Heparin.**

Sylvén, B., Acta Radiol., 1940, **21**, 206–212 has followed this matter up by subjecting rats and guinea pigs (in which the basophilic granules are said to be less soluble in water than in most other animals) to Gamma rays. He fixed the tissues in weaker aq. basic lead acetate (4%) for 24 hrs., stained paraffin sections with ½% aq. toluidin blue and other dyes, and reached the conclusion that the radiation brings about liberation of organic sulphuric acids of high molecular weight. It would be natural to investigate the relation if any between heparin and the basophilic granules in buffy coat of centrifuged human blood containing say 0.5% basophiles and in that of certain turtles in which the percentage is as high as 80 as well as in livers.

Another method of study is to investigate heparin in relation to the characteristic dissolution of basophiles 2 days after the intraperitoneal injection of egg albumen (Webb, R. L., Am. J. Anat., 1931–32, **49**, 283–334).

**Tissue Culture,** methods (Buchsbaum, R. and Loosli, C. G., Methods of Tissue Culture in Vitro, Univ. Chicago Press, 1936; Parker, R. C., Methods of Tissue Culture, New York: Paul B. Hoeber, Inc., 1938, 292 pp.). Data on tissue cell colonies in vitro (Mayer, E., Tabulae Biologica, Den Haag: W. Junk, 1939, **19** (1), 65–275). Application to embryology, growth of bones, teeth, etc. (Fell, H. B., J. Roy. Micr. Soc., 1940, **60**, 95–112). A comprehensive review of plant tissue cultures (White, P. R., Biol. Rev., 1941, **16**, 34–48). Tissue culture has several great advantages over other techniques for the study of living cells. (1) They can be examined at high magnification over long periods of time so that excellent moving pictures can be made. (2) Individual cell types can be grown in pure cultures separate from all others and their properties noted. (3) The fluid environments of the cells can be regulated and their influence on the cells determined. But the conditions are nevertheless artificial as compared with those of cell life *in vivo*, and, to obtain the best results, one must be an expert in the technique.

**Tissue Eosinophiles.** Demonstration is easy by the same techniques as for **Eosinophile Leucocytes.** In rabbits a marked increase of tissue eosinophiles can be produced in maxillary sinus mucosa by pilocarpinization. This attains a maximum in 5 min. and disappears after 24 hrs. (Nemours, P. R., Arch. Otolaryng., 1933, **17**, 38–42).

**Tissue Fluid.** All living cells of the body are aquatic. There is reason to think that the tissue fluids, which they inhabit, are not of uniform composition throughout the body but exhibit regional differences (Cowdry, E. V., Problems of Ageing, Baltimore: Williams & Wilkins, 1942, 583–625). Except when present in large amounts, these tissue fluids cannot be collected for chemical analysis. Consequently microchemical means are important in determination of their nature. They are often described in the literature as *intercellular ground substance.* Many methods have been described by S. H. Bensley (Anat. Rec., 1934, **60**, 93–109) for the ground substance of **Loose Connective Tissue.** See also **Cartilage** and **Bone.**

**Titan Yellow** (CI, 813)—Erie fast yellow WB, thiazole yellow—An acid thiazole dye used in fluorescence microscopy. See method for **Magnesium.**

**Titanium Dioxide.** Huggins, C., Anat. Rec., 1939, **74**, 231–253 used this compound

in a suspension as a vital stain for bone marrow because the amounts taken in by reticuloendothelial cells can be measured. He employed specially purified titanium chloride obtained from Dr. J. L. Turner and the Titanium Pigment Corporation, 111 Broadway, New York. The method is to make a fine 5% suspension in 2% aq. gum acacia by mixing with an electrical mixer for 1 hr. After keeping this at 4°C. for 2 days siphon off the supernatant fluid for use to avoid aggregates which settle to the bottom. Keep this likewise on ice but warm to body temperature before intravenous injection. Inject slowly into ear veins of rabbits, each animal to receive 3–6 injections of 10 cc. on consecutive days. The titanium dioxide particles can easily be recognized as a black accumulation in the phagocytes and its amount can be determined chemically in fairly large bone samples by a method detailed by the author.

**Toluene Red.** Dimethyldiamidotoluphenazin. *See* **Platelet** staining solutions.

**Toluidin Blue O** (CI, 925)—methylene blue T 50 or T extra—Employed very widely.

**Toluidine Blue Phloxinate.** Instructions for preparation (Lillie, R. D., Stain Techn., 1941, **16**, 1–6). Mix 10 gm. toluidine blue (62% dye content) dissolved in 500 cc. aq. dest. with 10 gms. phloxine B (82% dye content) likewise dissolved in 500 cc. aq. dest. After leaving overnight collect ppt. on a hard filter paper in suction filter and dry. The filtrate should be very light clear blue showing almost complete combination of both dyes. Dissolve 0.3 gm. in 50 cc. C.P. neutral anhydrous glycerin overnight at 56°C. Cool and add 50 cc. neutral methyl alcohol to make stock solution. Bring paraffin sections to water and stain 1 hr. in: stock solution, 2 cc.; acetone C.P., 3 cc.; methyl alcohol C.P., 3 cc., McIlvaine buffer of desired value, 2 cc.; aq. dest., 30 cc. After formalin and Orth fixatives buffer should be chosen to give pH 4.2–4.6; after formalin-Zenker, pH 5.0 and after methyl alcohol, absolute alcohol or Carnoy's fluid pH 6.5. See **McIlvaine Buffers.** Rinse in water, dehydrate in acetone and, after passing through equal parts acetone and xylol, clear in 2 changes xylol and mount in clarite. This gives fine colors, excellent metachromatic staining of mast cell granules and coloration of nuclei and bacteria.

**Toluylene Blue** (CI, 820). A basic indamin dye, homologue of **Bindschelder's Green** which see.

**Toluylene Red,** see **Neutral Red.**

**Tony Red,** see **Sudan III.**

**Torulosis,** see **Blastomycosis.**

**Toxic Neutrophiles** (*see* Neutrophiles, toxic).

**Trachea.** Excellent experimental methods to demonstrate secretion of **Mucus** are detailed by Florey, H., Carleton, H. M. and Wells, A. Q., Brit. J. Exper. Path., 1932, **13**, 269–284. Techniques for **Nerve Endings** are given under this heading but it would be helpful to consult Larsell, O. and Dow, R. S., Am. J. Anat., 1933, **52**, 125–146 who illustrates what one may expect to find. Techniques for **Cilia** require no special adaptation. Celloidin sections are smoother than paraffin ones.

**Trachoma Bodies.** These are easily colored by Giemsa's stain. For demonstration of glycogen in them and other pertinent data see Thygeson, P., Am. J. Path., 1938, **14**, 455–462.

Evolution forms of *Rickettsia trachomatis*. Fix smears in iodine alcohol, 4–5 min. Stain in May-Grünwald, 1 part; Giemsa, 1 part; neutral aq. dest. 10 parts for 1 hr. Differentiate in 95% alcohol (Foley, H. and Parrot, L., Arch. Inst. Pasteur d'Algérie, 1938, **16**, 283–292). See colored plates by the authors.

**Transplantation.** This technique provides opportunities for important microscopic studies. See **Tooth Germs, Anterior Chamber of Eye.**

**Trematodes.** Make up stain by mixing 1 gm. of dried residue on filter paper from Schneider's **aceto-carmine** with 10 gm. ammonia alum in 200 cc. aq. dest. with aid of heat. When dissolved, cool, filter and to filtrate add crystal of thymol. After fixation bring worms to water or to 20% alcohol. Stain 12–36 hrs. depending on size. Remove to water 2 changes. Dehydrate through 20, 35, and 50 to 70% alcohol. Place few crystals potassium chlorate in small glass covered dish; add few drops conc. HCl. When chlorine is given off fill dish with 70% alcohol. If deeply stained differentiate in this chlorinated alcohol. If not or the specimens are small ones add it to the alcohol covering them and agitate. When sufficiently destained remove to fresh 80% alcohol. Dehydrate in alcohol. Add cedar wood oil to the absolute until mixture is one half oil. Clear in cedar oil and mount in balsam (Gower, W. Carl, Stain Techn., 1939, **14**, 31–32).

**Treponema Pallida.** The organisms can best be seen in the primary lesions by **Darkfield** examination. The same method is useful for skin and lymph nodes in the secondary stage but for the tertiary lesions in deep lying tissues sections are desirable supplemented by smears. A negative finding is comforting but does not necessarily signify

absence of parasites unless confirmed serologically.

1. Low surface tension stain for *smears* (Haire, R. D., J. Lab. & Clin. Med., 1938, **23**, 1215–1216). Mix 1 gm. Gentian violet (or crystal violet) in mortar slowly adding 100 cc. hexylresorcinol. Filter and store filtrate in stock bottle. Stain smears 30 min. Wash in water, dry and examine. Stain on slide must not be heated. Treponemas, light purple.

2. **Wright's** stain for *smears* (Mallory, p. 289). To make stain add 1 cc. **Wright's** stain and 1 cc. 1% aq. potassium carbonate to 10 cc. aq. dest. in test tube and heat to boiling. Spread material thinly on cover glass (not slide) and hold level with forceps. Cover with hot stain 3–4 min. After fluid has turned violet, and a yellow metallic scum has formed over it, pour off and repeat process twice with hot stain. Wash in water, dry and mount in balsam. Treponemas, intensely violet.

3. **Giemsa's** stain for *smears* (Giemsa, G., Deut. med. Wochn., 1909, **35**, 1751–1752) after Mallory (p. 290). Fix smears for 15 min. in absolute alcohol or pass them through flame thrice. Pour on freshly diluted stain (1 cc. aq. dest. + 1 drop stock Giemsa). Steam gently and leave 15 sec. Decant and add immediately fresh diluted stain, warm and let cool 15 sec. Repeat 4 times leaving 1 min. last time. Rinse quickly in running water. Blot. Mount in balsam. Treponemas, dark red.

4. Fontana-Tribondeau silver method for *serum* (Fontana, A., Dermat. Zeits., 1925–26, **46**, 291–293) after Mallory (p. 291). To make silver solution add ammonia water (diluted 1:20) drop by drop to 50–100 cc. 1% aq. silver nitrate until a coffee colored clouding takes place. Air dry thin smears of serum. Pour on few drops Ruge's sol. (aq. dest., 100 cc.; glacial acetic, 1 cc.; formalin, 2 cc.) and change several times during 1 min. Rinse in running water. Mordant with a little aq. dest., 100 cc.; tannic acid, 5 gm.; liquid carbolic acid, 1 cc. for 20 sec. warming to steaming. Rinse in aq. dest. Treat with silver solution 30 sec. heating slightly. Wash in tap water. Dry in air. Mount in balsam. Treponemas, brown to deep black.

5. Burri's India Ink method for *lesion fluid* (Mallory, p. 291). Make 1:4 suspension of India ink in aq. dest. Sterilize in autoclave, 15 min. Mix this in equal parts with fluid from lesion on slide with platinum loop. Spread thinly. Dry and examine. Treponema (and bacteria if present), white in brown to black background.

6. Quick method for demonstration in *fresh autopsy tissues*. This is Krajian's modification of Dieterle's method (Am. J. Syphilis, 1933, **17**, 127) as amplified in Stain Techn., 1935, **10**, 68. Fix tissue 5 mm. thick 10 min. in 10% formalin, 70°C. Cut frozen sections 5–7 microns. Place in 2% aq. sodium cobalti nitrite 5 min. Wash 2 changes aq. dest. Mordant for 15 min. at 70°C. in uranium nitrate 1 gm.; 85% formic acid, 3 cc.; glycerin, 5 cc.; acetone, 10 cc.; 95% alcohol, 10 cc. Wash quickly in aq. dest. Develop 5 min. in 10 cc. of following mixture + 1 drop albumin-glycerin before use (hydroquinone, 0.62 gm.; sodium sulfite, 0.12 gm.; acetone, 5 cc.; 40% neutral formaldehyde, 5 cc.; pyridine, 5 cc.; sat. gum mastic in 95% alcohol, 5 cc., aq. dest., 30 cc.). Wash few sec. aq. dest. Then warm silver solution 15–25 sec. and wash in aq. dest. Keep all solutions in cool place. (Original gives treatment with 0.75% aq. silver nitrate at 70°C for 1 hr. upon the development in hydroquinone mixture.)

7. Levaditi's *block silver* method (Mallory, p. 293). Fix tissue pieces (1 mm. thick) in 10% formalin, 24 hrs. Rinse in aq. dest. 95% alcohol, 24 hrs. Transfer to aq. dest. and leave until tissue sinks to bottom. Fresh 1.5–3% aq. silver nitrate at 37°C. in dark 3–5 days changing 3 times. (The stronger silver is advised for tissues excised during life.) Wash in aq. dest. Reduce 24–72 hrs. in dark at room temperature in: aq. dest., 100 cc.; formalin, 5 cc.; pyrogallic acid, 2–4 gms. Wash in aq. dest. Dehydrate in 80, 95 and absolute alcohol. Clear in oil of cedar wood, imbed in paraffin, mount 5$\mu$ sections on slides, remove paraffin and mount in balsam. Treponemas, black.

8. Heitzman's modification of the Warthin-Starry and Nieto's methods as given by Mallory (p. 293). Cut *frozen sections* 15$\mu$ or less of 10% formalin fixed tissue. Place directly in pyridine, 10 min. Wash in aq. dest., 3 changes. 1% aq. uranium nitrate at 37°C., 15 min. Wash quickly in aq. dest., 2 changes. 0.25% aq. silver nitrate at 56°C., 15–30 min. Develop until dark brown in following mixture made immediately beforehand by pipetting into a beaker: (1) 15 cc. 5% aq. gelatin at 56°C.; (2) 3 cc. 2% aq. silver nitrate; (3) 0.5 cc. 1% aq. hydroquinone. Remove and thoroughly wash in warm aq. dest. Dehydrate on slide adding by pipette increasing alcohols to absolute. Clear in benzol and mount in balsam.

A heavy black ppt. indicates too long development. Treponemas, black.

9. For routine paraffin sections, Steiner, G., J. Lab. & Clin. Med., 1939, **25**, 204–210. Fix in 10% formalin and make sections 9–10 microns. Remove paraffin with xylol. Pass through 2 changes abs. alc. Treat 1–1½ min. in 4% uranium nitrate in abs. alc., 20 cc.; 25% gum mastic in abs. alc., 40–50 cc.; abs. alc., 20–30 cc. Wash in at least 3 changes aq. dest. until streaks of gum mastic are removed. 0.1% aq. silver nitrate in water bath at 100°C., 1–1½ hrs. Wash in aq. dest. Then through 80% and 95% to abs. alc. 10–12.5% gum mastic in abs. alc. 5 min. Repeat 3 washings described in aq. dest. Reduce 20–30 min. in: hydroquinone, 10 gm.; 12.5% gum mastic in abs. alc., 1 cc.; aq. dest., 200 cc. (with temperature gradually raised to 100°C.). Wash thoroughly in aq. dest. Counterstain with hematoxylin and eosin if desired. Dehydrate in abs. Clear in xylol and mount in balsam. The advantages are speed and decrease in confusing silver deposits. See Steiner's illustrations.

**Triacid Blood Stain,** see **Ehrlich's.**

**Tri-Amino Tri-Phenyl Methane Dyes.** These are the rosanilins. Examples: acid fuchsin, acid violet, anilin blue WS, basic fuchsin, benzyl violet, crystal violet, ethyl green, ethyl violet, Hofmann's violet, iodine green, isamine blue, magenta II, methyl blue, methyl green, methyl violet, new fuchsin (magenta III), pararosanilin (magenta O), rosanilin (magenta I), spirit blue, victoria blue B and R and victoria blue 4R.

**Trichinella Spiralis.** Mallory (p. 304) gives as a rapid method of diagnosis the squeezing of small pieces of jaw muscle or of muscle near tendon of diaphragm between two slides and direct examination at low magnification. If trichinellae are calcified or encapsulated specimens can be cleared with acid. In permanent preparations of Zenker or formalin fixed material stained with hematoxylin and phloxine or eosin the parasites are best seen in longitudinal sections of muscle fibers. To demonstrate in migratory phase withdraw blood from vein in arm into syringe containing 3% aq. acetic acid, centrifuge and examine.

Rapid iodine-silver technique (Kalwaryjski, M. B. E., Wojsk. Przegl. Weteryn., 1938, **9**, 123–136). Place thin slices of muscle for 10 min. in iodine, potassium iodide, aq. dest. sol. in following proportions 2:4:100 or 0.5:1:100 or 0.1:0.2:100. Wash in aq. dest. Destain in 2.5% aq. sodium thiosulphate until muscle is clear. Wash in aq. dest. Equal parts 10% aq. silver nitrate and strong ammonia until iodine leaves parasites. Wash in aq. dest. Decolorize in 5% aq. sodium thiosulphate. Wash in aq. dest. and mount in glycerin. Parasites stained dark brown owing to conversion of iodine to silver iodide.

See investigation of larvae with radioactive phosphorus (McCoy, O. R., Downing, V. F. and Voorhis, S. N., J. Parasit., 1941, **27**, 53–58).

**Trichloracetic Acid** employed with mercuric chloride and acetic acid as a fixative (Heidenhain, Zeit. wiss. Mikr., 1909, **25**, 405) also used in 4 or 5% aq. sol. as decalcifying agent.

**Trichlorethylene,** as a solvent in histological technique in place of xylol (Oltman, R. E., Stain Techn., 1935, **10**, 23–24).

**Trichlorlactic Acid** used as fixative followed by staining with resorcin fuchsin for cytoplasmic canalicular apparatus (Holmgren, E., Ergeb. d. Anat., 1901, **11**, 274–329; Cowdry, E. V., Internat. Monatsschr. f. Anat. u. Physiol., 1912, **29**, 1–32).

**Trimethylcarbinol,** see **Tertiary Butyl Alcohol.**

**Tropaeolin D,** see **Methyl Orange.**

**Tropaeolin G,** see **Metanil Yellow.**

**Tropaeolin G** or **OOO No. 1,** see **Orange I.**

**Tropaeolin OOO No. 2,** see **Orange II.**

**Trypan Blue** (CI, 477)—azidine blue 3B, benzamine blue 3B, benzo blue 3B, chlorazol blue 3B, Congo blue 3B, dianil blue H3G, naphthamine blue 3BX, Niagara blue 3B—This acid disazo dye is the most popular of all **Vital Stains.** See also trypan blue capillary permeability test (e Silva, M. R., and Dragstedt, C. A., J. Pharmac. and Exper. Therap., 1941, **73**, 405–411).

**Trypan Red** (CI, 438). So named because of influence on Trypanosome infections (G. *trypanon*, anger + *soma*, body). An acid dis-azo dye much used as a vital stain but less satisfactory than trypan blue.

**Trypsin,** a gelatin plate method as described under **Pepsin** but slightly modified is recommended.

**Tryptophan,** see **Romieu Reaction.**

**Tubercle Bacilli.** Stain by **Carbol Fuchsin,** see **Acid Fast Bacilli.** See **Concentration** method for sputum. Fluorescence with auramine has been described (Hagemann, P. K. H., Münch. med. Woch., 1938, **85**, 1066). Fix smears by flame and stain 15 min. in 1:1000 aq. auramine (Bayer) containing 5% phenolum liquefactum (liquid carbolic acid). Wash in tap water. Decolorize in ethanol 100 cc.; HCl conc., 4 cc.; sodium chloride, 4 gm. renewing solution after 1½ min. Wash thoroughly in tap water.

Examine without cover glass under fluorescence microscope using apochromatic dry objective and 3 compensating ocular (× about 180). For visible and red rays employ 3.5 mm. "Uvet" lens and 2% aq. copper sulphate. Bacilli, golden yellow rods in violet fluorescent background.

**Tungstic Acid,** a stable solution (Abrahamson, E. M., Tech. Bull., 1940, **1**, 75).

**Turnbull Blue** reaction for iron. Same as Berlin blue except use K ferricyanide and HCl.

**Turpentine.** Not advised as clearing agent. See test for **Alcohol** absolute.

**Tyrode** solution. NaCl, 0.8 gm.; KCl, 0.02 gm.; $CaCl_2$, 0.02 gm.; $MgCl_2$, 0.01 gm.; $NaH_2PO_4$, 0.005 gm.; $NaHCO_3$, 0.1 gm. (giving pH about 7.5–7.8); dextrose, 0.1 gm.; aq. dest., 100 cc. Solution cannot be boiled but can be passed through a Berkfeld filter.

**Ultracentrifuge,** see **Centrifugation.**

**Ultramicroscope,** see **Darkfield.**

**Ultraviolet Photomicrography** has certain advantages over visible light photomicrography because the resolving power of the former is greater in consequence of its shorter wave length, and as pointed out by Wyckoff and Louw (R. W. G. and A. L., J. Exper. Med., 1931, **54**, 449–451), because some proteins absorb ultraviolet more strongly than others, details can be brought out with it not revealed by visible light. This they demonstrate by experiments with *B. subtilis*. It was then found that the substances that strongly absorb ultraviolet light give a positive Feulgen reaction (Wyckoff, R. W. G., Ebeling, H. H., and Ter Louw, A. L., J. Morph., 1932, **53**, 189–199) and that they also yield conspicuous mineral ash on microincineration (Scott, G. H., Science, 1932, **76**, 148–150)—an interesting superposition of three technical methods.

**Unna's Orcein** method for elastic fibers. This is simple and direct. Stain paraffin sections, after almost any fixation, in: orcein, 1 gm.; absolute alcohol, 100 cc.; and hydrochloric acid, 1 cc. for several hours. Wash in 70% alcohol and sharpen the deep brown coloration of the elastic fibers by removing excess stain from background by destaining under the microscope in 95% alcohol plus a trace of hydrochloric acid. Wash in 95%, dehydrate, clear and mount. If desired counterstain with methylene blue.

Dahlgren (McClung, p. 425) advises a modification of this stain for **Muscle.** After sublimate fixation stain sections 24 hrs. in Wasserblau, 0.25 gm.; absolute alcohol, 60 cc.; orcein, 1 gm.; glycerin, 10 cc.; water, 30 cc. Wash in 70% alcohol, dehydrate, clear and mount. Muscle, purple; collagenic fibers, blue; elastic fibers, red. It is important in doubtful cases to compare with similar tissue colored by other specific stains before identification of muscle is assured.

**Uranin,** sodium salt of **Fluorescein.**

**Uranium.** Salts injected into tissues can be demonstrated by (1) a method of Schneider (G., Skand. Arch. Physiol., 1903, **14**, 383–389). Fix in: 5% aq. potassium ferrocyanide, 50 cc., sat. aq. picric acid, 50 cc.; hydrochloric acid, 10 cc. Wash in 4% aq. hydrochloric acid and then in 80% alcohol acidified with hydrochloric acid. Imbed and cut. The uranium ferrocyanide of potassium is detected by its dark brown color (Lison, p. 103). (2) the Prussian blue reaction for iron as employed by Gérard and Cordier (P. and R., Arch. Biol., 1932, **43**, 367–413). According to Lison this method is highly specific.

The possibility of detecting uranium salts in incinerated sections by their fluorescent properties in ultraviolet light has been described (Policard, A. and Okkels, H., Abderhalden's Handb. d. biol. Arbeitsmethoden, 1931, **5**, 1815). Gordon H. Scott has been successful when large amounts are present but has called attention to complicating factors (McClung's Microscopical Technique, p. 660).

**Urates and Uric Acid.** A modification of Courmont-André's method is suggested. Neutralize some formalin with calcium carbonate. Fix tissue in equal parts 1% aq. silver nitrate and 4.4% neutral formalin in darkness, 12–24 hrs. Wash in several changes aq. dest., 24 hrs. Imbed in paraffin. Stain sections hematum 10 min.; running tap water ½–1 hr.; 1% aq. orange G or eosin ½–1 hr. Wash quickly in aq. dest. Place in 0.5% aq. phosphomolybdic acid, rinse in aq. dest. and color in 0.12% aq. light green, 1–10 min. Differentiate quickly in 96% alcohol, dehydrate in iso-amyl-alcohol, clear in xylol and mount in balsam. Urates, black; chromatin, blue; protoplasmic inclusions red to orange and collagenic fibers, green. Employed by Hollande for bacteriocytes of *Periplaneta orientalis L* (Hollande, A. C., Bull. d'Histol. Appl., 1931, 8, 176–178).

**Urea.** Many histochemical techniques have been proposed. Leschke (E., Zeit. Klin. Med., 1915, 81, 14–35) fixes in half sat. sol. mercuric nitrate in 1% nitric acid for 1 day, then washes in frequently changed aq. dest., imbeds

in paraffin and treats the sections with sat. aq. hydrogen sulphide staining nuclei with hemalum. Stübel (H., Anat. Anz., 1921, **54**, 237–239) fixes small pieces in 6% xanthydrol in glacial acetic acid 6–12 hrs., imbeds in paraffin, stains sections by ordinary methods and examines by polarizing microscope. Oliver (J., J. Exper. Med., 1921, **33**, 177–186) employs instead a solution containing 2 gm. xanthydrol, 10 cc. methyl alcohol and 20 cc. glacial acetic acid. Lison (p. 169) criticizes these methods severely.

**Urease.** A method for determining the distribution of urease in the gastric mucous membrane (pylorus and fundus) of the dog has been described and used by Linderstrøm-Lang and Ohlsen (K. and A. S., Enzymologia, 1936–37, **1**, 92–95). Cylinders of tissue (2.5 mm. in diameter) are cut vertical to the surface from frozen mucosa. Cross frozen sections (25 microns thick) of the cylinders are then tested for urease. This is concentrated in the surface layers containing cells stainable with mucicarmine. Chief cells in the bases of the glands are inactive in both fundus and pylorus and the authors think it very unlikely that the parietal cells contain urease.

**Urinary Casts,** staining with methyl blue picric acid. To sediment from centrifuged urine add 1 drop 0.5% aq. eosin. Mix by side to side shaking. After 1–2 min. add 2 drops from 1 cc. 1% aq. methyl blue + 10 cc. sat. aq. picric acid and again mix. Color of sediment should be distinctly bluish green. If it is reddish brown add more methyl blue-picric acid. Transfer to slide cover and examine. The casts should be distinct blue but not too dark. Numerous details are brought out (Behre, J. A. and Muhlberg, W., J. Lab. & Clin. Med., 1936–37, **22**, 853–856). See the author's figures.

**Urinary Sediments.** The following outline is from Stitt (pp. 707–713) much abbreviated. Concentrate sediment by centrifuging 15 cc. fresh urine 1500 r.p.m. 5 min. but not longer. Decant supernatant urine. Suspend sediment in 2 cc. urine as is the practice in the Naval Medical School. By always using these amounts quantitative differences from normal in individual sediments become apparent. Examine for epithelial cells, leucocytes, erythrocytes, casts, crystalline materials, bacteria and so forth.

**Vaccinia,** Cytoplasmic inclusions in, see Cowdry, E. V., J. Exper. Med., 1922, **36**, 667–684.

**Vaginal Smears.** On the basis of large experience Papanicolaou G. N., J. Lab. & Clin. Med., 1940–41, **26**, 1200–1205 has described techniques in detail.

1. Fix immediately (before drying) in equal parts 95% alcohol and ether 1–2 min. Rinse in 70%, 50% alcohol and in aq. dest. Ehrlich's hematoxylin (or other hematoxylin), 1–2 min. Rinse in aq. dest. Rinse few times in 1% hydrochloric acid (may be omitted). Running water, 5 min., or aq. dest. 100 cc. + 3 drops conc. aq. lithium carbonate, 1 min. Do not leave slides too long in running water. Rinse in aq. dest. and stain for 2 min. in any of 6 combinations of stains recommended. One of these is made up of National Aniline and Chemical Co. dyes in 0.5% aq. sol. as follows: Light green S. F. yellowish, 12 cc.; orange G, 24 cc., acid fuchsin, 20 cc., eosin yellowish, 40 cc. + phosphomolybdic acid (Merck) 0.45 gm. Rinse in water. Rinse in dioxan 10–15 times until smears are clear. Pass through absolute alcohol to xylol. Mount in clarite, balsam or dammar.

2. A shorter method is, after similar fixation of smears brought down to aq. dest., to stain them 2–3 min. in anilin blue, water soluble, 16 cc.; acid fuchsin, 23 cc.; orange G, 17 cc.; eosin yellowish, 44 cc., (all of 5% aq. solutions) + phosphomolybdic acid 0.2 gm., and phosphotungstic acid, 0.2 gm. Rinse in water. Rinse in dioxan until clear, then through absolute alcohol and xylol to clarite. Nuclei, red; erythrocytes, orange; cornified cells, red, pink or orange; basophile cells, green or blue. Nuclei not as dark and cell outlines as sharp as after hematoxylin, but cornified cells are more prominent and basal cells more transparent.

**Valves.** Aortic, staining of elastic tissue in (Wilens, S. L., Arch. Path., 1940, **29**, 200–211). X-ray demonstration of valves of veins (Edwards, E. A., Anat. Rec., 1936, **64**, 369–385).

**Van Gehuchten's** mixture, see **Carnoy's Fluid.**

**Van Gieson's Connective Tissue Stain.** Paraffin sections of Zenker fixed material are stained with Harris' hematoxylin. Rinse in water. Stain in 1% aq. acid fuchsin 7.5 cc. and sat. aq. picric acid 50 cc., 10 min. Wash in 95% alc., dehydrate, clear and mount. Muscle yellow, collagenic fibers red, nuclei blue black. A brilliant stain. But it fades quickly and is not so much employed at present as Mallory's connective tissue stain. See **Buzaglo's Method.**

**Van Wijhe's** method for staining cartilage in whole tissues with methylene blue. See **Cartilage.**

**Vasa Vasorum.** Injection with India ink (Winternitz, M. C., Thomas, R. M. and LeCompte, P. M., The Biology of Arteriosclerosis. Springfield: Thomas, 1938, 142 pp.). Filter Higgins Engrossing ink through coarse filter paper and dilute filtrate with 8 times volume of aq. dest. Obtain pressure apparatus consisting of 2 liter metal tank with top and bottom outlets and air pressure gauge. Connect upper outlet with escape valve and high pressure air line and the lower one with rubber tube and cannulae. To inject vasa of coronary arteries place fresh human heart unopened in 0.9% aq. sodium chloride containing 0.1% sodium nitrite and a little thymol for 24 hrs. at 3-4°C. Just before injection warm heart to 37°C., tie cannulae in openings of coronary arteries and clamp or ligate all openings of heart except the aorta. By opening and closing the escape valve the ink in the tank is driven into the coronaries by a pulsating pressure. During first 10 min. maintain the minimum pressure at about 100 mm. of mercury with maximum pressure of pulsations not more than 200. Then increase slowly so that during next 20 min. the minimum pressures vary 500–800 mm. and the maximum 800–1000. After injection put heart in 10% formalin for 24 hrs. Dissect out main coronaries. Clear by **Spalteholz Method** for whole mounts or imbed in paraffin section and color by **Masson's Trichrome** stain. The authors give special directions for injecting the aorta and vessels of kidneys and amputated legs. Their illustrations afford useful guides to the results expected.

**Vaseline** in tissues can be distinguished from the normal fats by the fact that the former is colored clear violet and the latter intense blue black by staining for 15 min. with **Sudan Black B.** Terebenthine, turpineol and methyl benzoate are colored blue black (Gerard, P., Bull. d'Hist. Appl., 1935, **12**, 92–93).

**Veins,** see **Blood Vessels** and a very fine presentation by Franklin, K. J., A Monograph on Veins. Springfield: Thomas, 1937, 410 pp. with hundreds of references to techniques and results.

**Venous Sinuses,** splenic, direct observation *in vivo* (Knisely, M. H. Anat. Rec., 1936, **64**, 499–524; **65**, 23–50). See **Spleen.**

**Venules.** A graphic demonstration of venules in the ears of white mice can be obtained by intravenous injection of Chicago blue because this dye escapes into the surrounding tissue fluid more easily from venules than from capillaries (Smith, P. and Rous, P., J. Exper. Med., 1931, **54**, 499–514).

**Verhoeff's Elastic Tissue Method** (Verhoeff, F. H., J. A. M. A., 1908, **50**, 876–877). Gives good results after fixation in Zenker's fluid, formalin alone or after Weigert's mordant for myelin sheaths or Marchi's fluid. It is fairly satisfactory for tissues decalcified with nitric acid. Mercury deposits resulting from Zenker's fixation are removed by the stain: Hematoxylin crystals, 1 gm.; Abs. alc., 20 cc.; Dissolve in test tube with aid of heat, filter and add in order given: 10% aq. ferric chloride, 8 cc.; Conc. Lugol's solution (iodine, 2; potassium iodide, 4; water, 100), 8 cc. Stain sections in above sol. 5 min. or more. Differentiate in 2% aq. ferric chloride for a few sec. until the connective tissue takes the color of Lugol's solution. Keep sections in motion during differentiation. They can be examined at low magnification in water and if over differentiated can be restained at this stage. Wash in water followed by 95% alc. to remove the stain of Lugol's solution. Then leave in water 5 min. or more. Counterstain in 0.2% water sol. eosin in 80% alcohol. Dehydrate, clear in origanum and mount in balsam. Elastic tissue, black; fibroglia, myoglia, neuroglia, myelin and fibrin, pink. Degenerated elastic tissue (elacin) can be distinguished by less intensity of staining and by diffuse outlines.

*To differentially stain myelin sheaths* fair results are obtained after Zenker's fixative or formalin followed by Marchi's fluid. For best results fix in formalin 4 days, or longer, and mordant in Weigert's potassium bichromate and chrome alum for 4 days. Again it is not necessary before hand to remove mercurial precipitates. Place sections in 3% aq. potassium permanganate, 30 min. Wash in water and color for 30 min. in the hematoxylin stain described. Wash in water and differentiate in 10% aq. ferric chloride until the internal elastic membranes of blood vessels are decolorized as determined by examination in water at low magnification. 1–2 min. are required. Wash in water for 5 min., counterstain with eosin and mount in usual way.

**Vesuvin,** see **Bismark Brown Y.**

**Victoria Blue** (1) B (CI, 729)—corn blue BN, fat blue B—(2) R (CI, 728)—corn blue B, new Victoria blue B or R—(3) 4R (CI, 690)—fat blue 4R—A useful basic tri-phenyl methane dye. 4R is quite extensively discussed with other vital stains by Gutstein, M., Zeit. f. d. Ges. Exp. Med., 1932, 82, 479–524.

Herzberg, K., Zentralbl. Bakt. I Abt. Orig. 1934, **131**, 358–366 employed *4B* highly concentrated (Bayer standards, Hollborn), as a stain for filterable viruses (Kikuth, variola, varicella, ectromelia and possibly herpes). Dry smears in air 24 hrs. Stain 5–20 min. in 3% aq. Victoria blue. This dye solution should have been heated to 60°C. for half an hour, allowed to stand 2 weeks and filtered before use. To increase intensity of stain add 0.3 cc. 10% aq. tartaric acid to 10 cc. of stain. Response of different viruses to stain is not uniform. Various counterstains are suggested. The various Victoria blues are not easily disentangled. Victoria blue (variety unspecified) has, according to Lee (p. 187), a special affinity for elastic fibers and mucous cells.

**Victoria Green B** or **WB**, see **Malachite Green.**

**Victoria Green G** (British Drug Houses Ltd), a triazo dye of benzidine series. In alcoholic solution gives blue green and yellow green colors. Can be used with Marshall red or Hickson purple (H. G. Cannan, J. Roy. Micr. Soc., 1941, **61**, 88–94).

**Victoria Rubin O,** see **Amaranth.**

**Villi,** method for study of movements (King, C. E. and Arnold, L., Am. J. Physiol., 1922, **59**, 97–131; King, Arnold and Church, J. G., *ibid*, **61**, 80–92). See **Agonal Changes.** Changes in shape when intestine is distended (Johnson, E. P., Am. J. Anat., 1912–13, **14**, 235–250).

**Vincent's Angina,** staining of spirochete. Spread ulcerative material on clean slide. Dry in air and fix with heat. N/20 HCl, 10 sec. Running water, 5 sec. Cover with Gram's iodine solution, 5–10 sec. Wash. Cover with anilin gentian violet, 5–10 sec. Wash. Gram's iodine, 5–10 sec. Wash. Anilin gentian violet, 5–10 sec. Wash, blot and examine. Spirochetes deep violet color. Also good for *T. pallidum* (Bailey, H. D., J. Lab. & Clin. Med., 1937–38, **23**, 960).

**Violamin 3B,** possibly related to fast acid blue.

**Violet R, RR** or **4RN,** see **Hofmann's Violet.**

**Viruses** may now be studied microscopically in several different ways. There is a general but not very satisfactory distinction made between **Elementary Bodies** of the viruses which may be extracellular and the **Inclusion Bodies** which may be larger, are intracellular and may contain cellular material perhaps combined with virus. The **Chorioallantoic Membrane** has proved to be an excellent tissue in which to examine virus action. See further data under above headings.

**Viscosity.** According to Heilbrunn (L. V., An Outline of General Physiology. Philadelphia: Saunders, 1937), "Viscosity can be roughly defined as the force which tends to hold the particles of a substance together when a shearing force acting on the substance tends to pull it apart." Viscosity is the inverse of fluidity. It is of great importance to histologists to be able to detect and if possible to measure changes in viscosity. When a living cell is examined in approximately an isotonic medium and tiny particles in it begin **Brownian Movement** a decrease in viscosity is indicated and when the movement ceases an increase is to be expected. Thus Lewis (W. H., Bull. J. Hopkins Hosp., 1923, **34**, 373–379) took cessation of Brownian movement of particles in the nucleus viewed in the dark field to mean gelation which is increase in viscosity. A **Microdissection** method is to insert 2 microneedles into a cell. If they can be pulled apart easily the viscosity is low; if with difficulty, it is high. The idea back of the **Ultracentrifuge** method is that if two cells of the same sort are subjected to equal centrifugal force and a component, say the nucleus, is displaced more in one than in the other the viscosity of the cytoplasm is greater in the cell showing the least nuclear displacement. But this is not necessarily true. One has to be sure that the nuclei are of equal **Specific Gravity.** If the more displaced nucleus is of higher specific gravity than the other it will be more subjected than the other to the centrifugal force and its greater displacement will not signify a lower viscosity of the surrounding cytoplasm. Similarly if the specific gravity of the cytoplasm surrounding the more displaced nucleus is less than that in the other cell the greater displacement subjected to the centrifugal force of the nucleus through it will not indicate a lower cytoplasmic viscosity. When a material changes from a sol to a gel its viscosity increases without a change in specific gravity. Consequently in the interpretation of alterations in displaceability of cellular components subjected to centrifugal force one has to be on the lookout for changes in specific gravity and colloidal state. For details in respect to intranuclear viscosity, see Cowdry, E. V. and Paletta, F. X., Am. J. Path., 1941, **17**, 335–357; 1942, **18**, 291–311).

**Vital New Red.** This is an acid dis-azo dye not listed in indexes but Conn (p. 64) calls attention to chlorazol fast

pink 4BL (CI, 353) as most nearly resembling it. Vital new red is one of the many dis-azo dyes employed by Evans, H. M., and Scott, K. J., Carnegie Inst. Wash., Contrib. to Embryol., 1921, **10**, 1–56 to bring out a difference in reaction of the two great groups of connective tissue cells.

**Vital Red** (CI, 456)—acid Congo R, azidine scarlet R, brilliant Congo R, brilliant Congo red R, brilliant dianil red R, brilliant vital red—An important acid dis-azo dye frequently used in standard method for determination of blood volume.

**Vital Staining.** This technique has been contrasted with **Supravital Staining.** It must be viewed broadly. Any nontoxic coloration of the living body is vital staining. It is not restricted to particulate materials or to colloidal suspensions which are phagocytosed by certain cells. The fat depots of an animal become vitally stained red when the said animal is fed fat colored with alcohol soluble sudan III. Bone formed while madder is available in the circulation is stained red and dentin is vitally stained violet by intravenous injections of 1% sodium alizarin sulphonate (Gottlieb, B., Ztschr. f. Somat., 1913, **11**, 452). The phthalein indicators tint the tissues of living animals faintly but almost all the colors of the rainbow. Bile capillaries of the liver can easily be stained by intravenous injection of sodium sulphindigotate. Many other examples of similar phenomena could be cited. But it is customary to think of vital stains as substances which are regularly taken in by cells of the **Reticulo-Endothelial System** and by a few others on occasion. These include colloidal suspensions of various benzidine dyes (trypan blue, isamin blue, pyrrhol blue, trypan red, etc.), of silver, Higgins ink, lamp black etc.; and of simple suspensions of India ink, carmine, graphite and so on. They are injected intravenously, intraperitoneally or subcutaneously. The literature is enormous. Consult latest issue of the Quarterly Cumulative Index Medicus. For chemistry of Benzidine dyes see Evans, H. M. and Schulemann, W., Science, 1914, **39**, 443.

The following experiment is suggested. Give each of a dozen or more white mice 1 cc. of 0.5% trypan blue in sterile aq. dest. intraperitoneally and in the course of a few minutes the beginning of deposition of the dye in the ears will be noted. Give similar doses every second day for 8 days. A few hours after the last draw a little blood from the tail and observe that some of the monocytes have taken up the dye. Then autopsy the mice and study the distribution of the dye in the tissues. The skin, kidneys, adrenals, liver, spleen and bone marrow will be found quite deeply colored while the nervous system has escaped. The heaviest accumulation will be in the peritoneal cavity near the sites of injection and in the loose connective tissue everywhere. Examination of fresh mounts in physiological salt solution will reveal that the dye is concentrated within (1) the epithelial cells of the convoluted tubules of the kidney, of the adrenal and choroid plexus; (2) certain cells of the ovary and testicle; (3) the macrophages of loose connective tissue and especially of the spleen, liver, bone marrow, adrenals and lymph nodes—fibroblasts are colored less deeply; and (4) the "specific endothelia" of the five organs mentioned. If permanent preparations are desired fix in 10% formalin and imbed in paraffin.

Vital staining in the narrow sense is used for many purposes. (1) To identify phagocytic cells of the reticulo-endothelial system and to see how they behave in normal and pathological conditions. (2) To locate injured cells because some cells that do not ordinarily stain take up the dye when injured. (3) To influence the activity of R. E. cells by blocking them with particulate matter. This has not been very successful. See R. E. Blockade (Victor, J., Van Buren, J. R. and Smith, H. P., J. Exper. Med., 1930, **51**, 531–548). (4) To measure the absorption by membranes of particulate matter (Wislocki, G. B., Anat. Rec., 1921, **21**, 29–33). (5) To distinguish between malignant and non-malignant cells (Ludford, R. J., Arch. f. exp. Zellf., 1933, **14**, 42–55). (6) To determine pH of different organs and tissues by injection with phthalein indicators (Rous, P., J. Exper. Med., 1925, **41**, 739–759). (7) To identify calcium salts laid down (**Alizarin Red S** and **Madder**). See method for **Reticulo-endothelial system.**

It is sometimes very worthwhile to inject simultaneously three materials, for example Higgins' Ink intravenously, trypan blue or Niagara blue intraperitoneally, and lithium carmine intrapleurally (Foot, McClung, p. 116). An interesting experiment is to feed Sudan III or Scharlach (scarlet = Sudan IV) colored lipids. Make solution in olive oil (about 20%). Introduce by stomach tube into a cat. There is slight staining of fatty tissue within 24 hrs. and maximum in 3–7 days (Hadjioloff, A., Bull. d'Hist. Appl.,

1938, **15**, 81–98). Try also inducing cat to drink large amount of milk or cream colored with Sudan III or Sudan black, see colored illustrations of Gage and Fish (S. H. and P. A., Am. J. Anat., 1924–25, **34**, 1–81). *History* of vital staining (Conn, H. J. and Cunningham, R. S., Stain Techn., 1932, 7, 81–90, 115–119). See **Chorioallantoic Membrane, Carmine, Indigo-Carmine, Manganese Dioxide, Higgins' Ink, Protargol** (silver), **Lampblack, Leuco-Dyes, Nuclei, Titanium Dioxide, Thorium Dioxide, Copper, Platinum, Iron, Mercury, Lymphatic Vessels.**

**Vitamins.** Some vitamins are susceptible of microscopic localization. Deficiencies in most of them leave structural imprints in the tissues. A list may therefore be useful. Up-to-date information is usually given in Annual Review of Biochemistry. See papers by P. György and R. A. Morton in the review for 1942, **11**, 309–364 and 365–390. A useful background is provided by Sherman, H. C., Chemistry of Food and Nutrition, New York: MacMillan, 1941, 611 pp. For a summary of tissue changes in vitamin deficiencies see Wolbach, S. B. and Bessey, O. A., Physiol. Rev., 1942, **22**, 233–289.

A. Growth promoting, anti-infective and anti-xerophthalmic vitamin. $C_{20}H_{29}OH$, mol. wt. 286.4. The term vitamin A is also applied to its provitamins: alpha-, beta- and gamma, carotene and cryptoxanthin. There are two tests for this vitamin. (1) The *antimony trichloride test* is the basis of the **Carr-Price** reaction, which see, as applied to mitochondria of hepatic cells. When the mitochondrial fraction is separated and collected by **Centrifugation** the vitamin A can be easily measured in it as the Goerner's have done in their several investigations (A. and M. M., J. Biol. Chem., 1937–38, **122**, 529–538; *ibid*, 1939, **128**, 559–565). This test also has been employed for vitamin A in serum the colors being checked against alizarin solutions (Parker, R. C., Methods of Tissue Culture, New York: Hoeber, 1938, 292 pp.). According to Joyet-Lavergne, P., C. rend. Acad. d. Sci., 1935, **201**, 1219–1221, vitamin A can be demonstrated in the red blood cells of rays (marine fish) by the antimony trichloride test (2) *Green fluorescence.* Much work has been done on the identification of vitamin A within cells by its characteristic but short lived fluorescence in ultraviolet light. Popper, H., Proc. Soc. Exp. Biol. & Med., 1940, **43**, 133–136, 234–236 advises fixation of liver in 10% formalin and examination of frozen sections with **Fluorescence Microscope** within 24 hrs. The green fluorescence fades during irradiation especially in the Kupffer cells. The same fluorescence is found in epithelial cells of fascicular and glomerular zone of adrenals (but it is absent in adrenals of newborn infant), in the cells of the corpus luteum, interstitial cells of the testis and several others. Greenberg, R. and Popper, H., J. Cell. & Comp. Physiol., 1941, **18**, 269–272 report that vitamin A, gives "striking green and quickly fading" fluorescence and $A_2$ "faint yellow-brown and slowly fading."

B. Complex contains many factors.

*Nicotinic acid* and *nicotin amide.* An excellent chemical method for quantitative determination of nicotinic acid has been advocated by Dam, W. J. and Handler, P., J. Biol. Chem., 1941, **140**, 201–213, 755–762. As to tissue localization it is reported by the same authors that nicotinic acid exists as part of nucleotide molecules (see **Pentose Nucleotides**) but in muscle or renal cortex most of it occurs in some other form. The status of the fluorescent substances present in the urine of pellagrins is not clear (Najjar, V. A. and Cleckley, H. M., Proc. Soc. Exp. Biol. & Med., 1941, **48**, 413–414).

*Pantothenic acid.* Filtrate factor, Factor W., Anti-grey hair factor. Localization as between blood plasma and cells apparently is possible (Pearson, P. B., J. Biol. Chem., 1941, **140**, 423–426.

*Choline.* No histochemical method.

$B_1$. Thiamine hydrochloride; thiamin chloride, anti-BeriBeri or antineuritic vitamin; Aneurine, Betabion, Betatoxin, Oryzanin, Torulin. $C_{12}H_{18}$, $ON_4SCl_2$, mol. wt. 337.26. There is no microchemical test for thiamin but tissue analysis reveals the curious fact that the amount in the adrenal cortex of the bull is more than 7 times that in the medulla while in the cow the medulla contains 1.9 times as much as the cortex (Wright, L. D., *et al.*, Univ. Texas Publ., 1941, **4137**, 38–60).

$B_2$. Vitamin G, Riboflavin; Lactoflavin. $C_{17}H_{20}O_6N_4$, mol. wt. 376.19. No microscopic methods are available but a microbiological technique for riboflavin has been described by Snell, E. E. and Strong, F. M., Univ. Texas Pub., 1941, **4137**, 11–13 which György says results in satisfactory agreement with those secured in other ways. Riboflavin can now be determined by polarographic analysis (Lingane, J. J., and Davis, O. L., J. Biol. Chem., 1941, **137**, 567–574. Sherman (p. 373) states that the respiratory enzyme (Warburg's yellow

enzyme) is a combination of riboflavin phosphate and protein (see **Cytochrome**).

$B_{3, 4, 5}$. Data insufficient. See Sherman (p. 390).

$B_6$. *Pyridoxin*, adermin, antidermatitis vitamin. $C_8H_{11}NO_3$, mol. wt. 169.18. This essential nutrilite apparently occurs in tissues to a large extent in bound form. The trouble with microbiological methods of analysis is that it may be only incompletely extracted as noted by György.

C. Antiscorbutic vitamin, Cebione, Redoxon. $C_6H_8O_6$, mol. wt. 176.06. Bourne (Anat. Rec., 1936, **66**, 369–385) has made a critical study of cytological methods for the detection of vitamin C. The technique recommended is based on the assumption that the only substance other than vitamin C capable of reducing an acid silver nitrate solution in the dark is hydrogen sulphide "which is not by any means a common constituent of living tissue, if it occurs at all."

To demonstrate reduced vitamin C frozen sections of fresh tissue are treated with 5% aq. sol. of silver nitrate to which 5 cc. acetic acid is added for each 100 cc. for a few minutes. The vitamin C granules blacken. After washing in aq. dest. fat may be stained in a solution of Sudan III or Scharlach R in 90% alc. and the section cleared and mounted in glycerin.

To reveal both reduced and oxidized vitamin C is more difficult. Bourne advises: Fresh tissue is subjected to glacial acetic acid vapor for several minutes. Cut into very thin slices and put in atmosphere of hydrogen sulphide for 15 min. All vitamin C is thereby converted to reduced form. Remove hydrogen sulphide by keeping in partial vacuum for 10 to 30 min. followed by strong stream of nitrogen gas for 15 min. Treat with acid silver nitrate solution as described.

If there is reason to believe that glutathione inhibits the reaction Bourne suggests, after hydrogen sulphide treatment, to momentarily wash the section, then plunge into mercuric acetate solution for a few minutes, wash and apply acid silver nitrate solution. See Barnett, S. A. and Bourne, G., J. Anat., 1940–41, **75**, 251–264 for methods of demonstrating vitamin C in chick embryos.

Modification of Giroud and Leblanc silver method (Tonutti, E., Protoplasma, 1938, 31 (1), 151–158). Briefly wash tissue in 5.4% aq. levulose. 10% aq. $AgNO_3$ + 2 drops glacial acetic per cc., up to 30 min. Rinse in aq. dest. 15–30 min. 3% aq. $Na_2S_2O_3$, 15–30 min. Rinse in aq. dest. 15–30 min. All this in dark room with red light. Change to 70% alcohol and imbed in paraffin. Counterstain with "Kernechtrot" and light green.

D. According to Sherman (p. 430) there are probably at least 10 such substances having antirachitic potency of which 5 are (in 1940) recognized fairly clearly as chemical individuals, $D_2$ and $D_3$ being of great importance. Action is measured histologically by the **Line Test.**

$D_1$. is molecular compound of $D_2$ and luminsterol which is first product of irradiation of ergosterol with ultraviolet light.

$D_2$. Calciferol, $C_{28}H_{44}O$ produced by irradiation or ergosterol.

$D_3$. Antirachitic vitamin. $C_{27}H_{44}O$, mol. wt. 384.6. This is activated 7-dehydrocholesterol.

E. Antisterility vitamin, $\alpha$ Tocopherol, $C_{29}H_{50}O_2$, mol. wt. 430.4. The 2 other vitamin E factors are $\beta$ and $\gamma$ tocopherol.

F. The designation of vitamin F was originally applied to essential fatty acid but it has not been officially accepted. G = $B_2$.

H. This term as Sherman (p. 393) points out has been used in at least 3 ways. It is considered to be Biotin.

$K_1$ is the first form of the antihemorrhagic vitamin to be isolated by Dam (see Dam, H., Helv. chim. Acta, 1939, **22**, 310–313). It is 2 methyl-3-phytyl-1,4-naphthoquinone.

$K_2$ is the second isolated by Doisy, *et al.* (see Brinkley, S. B., MacCorquodale, D. W., Thayer, S. A. and Doisy, E. A., J. Biol. Chem., 1939, **130**, 219–234). It is the same except that there is a longer more unsaturated side chain. Neither of the two can be localized histologically but we may expect histological studies of their action.

M. An unknown factor said to be essential to the nutrition of monkeys (Day, P. L., *et al.*, J. Exp. Med., 1940, **72**, 463–477).

P. Permeability vitamin, citrin, said to be essential for maintenance of walls of small blood vessels. For a discussion of vitamin P as measured by capillary fragility see Rapaport, H. G., and Klein, S., J. Pediatr., 1941, **18**, 321–327.

**Volkonsky Method** for mitochondria. This is a complicated technique involving staining with anilin fuchsin, aurantia, methylene violet and azure II but can give splendid results. See original account (Volkonsky, M., Bull. d'hist. Appl., 1928, **5**, 220–222).

**Volume.** As explained by Danielli (Bourne, p. 39), cell volume is a function of the number of contained osmotically active

particles unless change is restricted by rigidity of the enveloping membrane. A satisfactory technique for measuring the volume of red blood cells is to determine photoelectrically light absorption of a suspension (Jacobs, M. H., Biol. Bull., 1930, 58, 104). The simplest way to obtain ratio for cytoplasmic and nuclear volumes is to outline nuclei and cytoplasms on kodaloid and determine the weights as has been recently done in carcinogenesis (Cowdry, E. V. and Paletta, F. X., J. Nat. Cancer Inst., 1941, 1, 745–759). The technique, of course, varies with structure involved, for example thyroid colloid (Stein, H. B., Am. J. Anat., 1940, 66, 197–211), fresh endocrine glands (Swinyard, C. A., Anat., Rec., 1939, 74, 71–78). To determine volume and cell numbers in small organs (Dornfeld, E. J., *et al.*, Anat. Rec., 1942, 82, 255–259). For influence on tissue volume of various methods of fixation, dehydration and imbedding, see Stowell, R. E., Stain Techn., 1941, 16, 67–83.

**Volume** measurements

1 liter = 2.1 U. S. pints (1.76 Imperial pints)
1 cc. = 16½ minims (17 minims B.P.)
1 gallon = 3.79 liters (1 Imperial gallon = 3.79 liters)
1 pint = 473 cc. (1 Imperial pint = 568 cc.)
1 fluid ounce = 29.5 cc. (1 fluid ounce B.P. = 28.4 cc.)
1 fluid drachm = 3.7 cc. (1 fluid drachm B.P. = 3.5 cc.)
1 minim = 0.065 cc.

**Volutin.** Spherical bodies in fungi, bacteria and other organisms (Taylor in McClung's Microscopical Technique, p. 221).

**Vulpian Reaction** named after a Parisian physician. Fresh slices of the adrenal immersed in dil. aq. ferric chloride show a green coloration of the chromaffin cells of the medulla. It is a test for tissues producing epinephrine. See: **chromaffin reaction** and **osmic acid.**

**Warburg's Respiratory Enzyme,** see **Cytochrome-Oxidase.**

**Washing.** The surplus of most aqueous fixatives is removed by washing the tissue in water. In the case of Zenker's fluid, for example, wash for 12–24 hrs. in running tap water. A convenient way is to cover the wide mouth of a bottle containing the tissue with gauze secured by an elastic band. Water from the tap is allowed to drop onto the gauze or better is led into the bottle through the gauze in a small glass tube. Most laboratories are provided with many such water carrying tubes. The water pressure should be so regulated that the tissue is not bumped about by the stream. However, almost equally satisfactory results can be obtained by the more tedious method of frequently changing the water. Osmic acid containing fixatives are to be washed in aq. dest. for about an hour. After Regaud's fixative the tissue is transferred to 3% aq. potassium bichromate without washing in water. Tissues fixed in alcoholic mixtures are to be briefly washed in alcohol before dehydration. For details about washing see the individual fixatives.

**Wasserblau,** see **Brazilin-Wasserblau.**

**Water Absorption** by slices of liver. The method has been standardized by Sperry and Brand (W. M. and F. C., Proc. Soc. Exp. Biol. & Med., 1939, 42, 147–150) and may prove useful in the investigation of other tissues.

**Water Blue** (Wasserblau), see **Anilin Blue.**

**Wear and Tear** pigment, see **Lipofuscin.**

**Weigert Method.** For myelin sheaths. Kultschitzky modification (Romeis, B. Taschenbuch der mikroskopischen technik, ii Auflage Section 999, p. 332). Fix in 10% formalin and mordant in **Müller's Fluid,** or in **Formalin Müller** or in **Weigert's Quick Mordant.** Bring paraffin or celloidin sections to water. Immerse in 3% aq. potassium bichromate or in Müller's fluid 12 hrs. Stain for 12–24 hrs. in: 10% hematoxylin in abs. alc. (1–6 months old), 10 cc.; aq. dest., 100 cc. Wash and destain in: aq. lithium carbonate, 100 cc.; 1% aq. potassium ferricyanide, 10 cc. until clear differentiation appears between gray and white matter. Wash, dehydrate and mount.

The following is provided by Dr. J. L. O'Leary: Mordanting in the Weigert procedure serves two purposes: (1) It renders the myelin sheath components insoluble in the fat solvents necessary to secure dehydration and imbedding. (2) It distributes the chromate ion in sufficient concentration in the myelin sheaths to ensure the formation of an adequate lake with hematoxylin in the subsequent staining procedure. If paraffin imbedding is to be used, it is absolutely necessary to carry block mordanting to the point where the myelin of all fibers has been rendered insoluble. For this reason paraffin imbedding of material to be used for Weigert staining should be restricted to small nerves and thin pieces of spinal cord, otherwise overhardening results. Here excellent results are to be achieved, the smallest fibers staining as completely as by the osmic acid method. Two methods are applicable to paraffin imbedded sections, the procedures for which are given subsequently. These are: the Kultschitzky

modification of the Weigert method and O'Leary's Brazilin method. All large blocks of brain or spinal cord should be imbedded in celloidin, the length of time in celloidin and the type of celloidin to be used being determined by the thickness of the sections desired. The following general rules apply to the block mordanting of material to be stained by the Weigert method:

1. If it is advisable to stain nerve cells and myelinated fibers in alternate sections, it is best to forego block mordanting in Müller's fluid. Formalin fixed blocks are imbedded directly in celloidin and alternate sections are stained by **Weil's Method** and the **Gallocyanin Technique.**

2. If only staining by a Weigert procedure is contemplated, the blocks may be mordanted in Müller's fluid for several weeks to several months depending upon the size of the block, imbedded in celloidin and stained by the Weigert-Pal method.

3. In special cases (cerebral cortex) the small myelinated fibers are stained completely with great difficulty. Blocks, premordanted or not, are sectioned in celloidin and the sections given long mordanting (one week to one month) in Müller's fluid. Stain by Kultschitzky modification of Weigert or **Weigert-Pal.**

**Weigert's Mordants.** (1) Primary, or rapid mordant: potassium bichromate, 5 gm.; Fluorchrome, 2 gm.; boiling aq. dest., 100 cc. (2) Secondary, or copper, or neuroglia mordant: boil 2.5 gm. Fluorchrome with 100 cc. aq. dest. Take away flame. When boiling has stopped, add 5 cc. glacial acetic acid, then 5 gm. finely powdered copper acetate. Stir vigorously until dissolved and cool.

**Weigert Pal Method.** For myelin sheaths (Dr. J. L. O'Leary, personal communication). Fix in 10% formalin, 1–2 wks. Wash in running tap water, 3 hrs. Mordant in Müller's fluid 1 wk. to 3 mo. depending on the size of block. Change Müller's thrice weekly at first, later once weekly. Wash in running tap water, 6–12 hrs. Imbed in celloidin. Cut sections 20–100 $\mu$ depending upon size of block and detail desired. 0.25% aq. chromic acid, 3–5 hrs. 3 changes aq. dest. 10% hematoxylin in abs. alc. ripened and diluted to 1% with aq. dest. prior to use, 12–24 hrs. 3 changes of aq. dest. Differentiate in Pal's fluid (oxalic acid, 1 gm.; potassium sulphite, 1 gm.; aq. dest., 200 cc.), alternating with 0.25% aq. potassium permanganate if differentiation is too slow. Wash in 3 changes aq. dest. Dehydrate in 2 changes 95% alc. Clear in carbol-creosol-xylol followed by pure toluol. Mount in balsam. Myelin sheaths, deep black; background, unstained. Another variation of the Pal-Weigert method is given by Clark, S. L. and Ward, J. W., Stain Tech., 1935, **10**, 53–55. See **Johnson's Neutral** red for counterstain.

**Weigert's Borax Ferricyanide.** Borax, 1 gm.; potassium ferricyanide, 1.25 gm.; aq. dest., 100 cc. A fluid for differentiation of hematoxylin stain in Weigert's method. Employed also in copper chrome hematoxylin method of Bensley.

**Weigert's Resorcin-Fuchsin.** Stain for elastic fibers. Given by Mallory, p. 168. Add 2 gm. basic fuchsin and 4 gm. resorcin to 200 cc. aq. dest. Boil in enamel dish and while boiling, add 25 cc. 29% aq. ferric chloride. Stir and boil 2–5 min. Cool. Collect ppt. and discard filtrate. Dry ppt. on filter paper and return both to the enamel dish which has also been dried. Add 200 cc. 95% alcohol, warm carefully, stir and discard filter paper when ppt. is dissolved out. Cool, add 95% alcohol to 200 cc. and 4 cc. hydrochloric acid. Mixture keeps well. Formalin fixed material is preferred, but most other fixatives are satisfactory. Stain paraffin sections, after removing paraffin, for 20 min. or more in above mixture. Wash off excess in 95% alcohol and differentiate in **Acid Alcohol** if required. Wash thoroughly in tap water. Dehydrate, clear and mount. Elastic fibers dark blue black. It is well to stain nuclei with **Lithium Carmine** (Orth's) before coloring the elastic tissue.

**Weight** measurements

1 kilogram = 2.2 lbs., or 35½ ounces
1 gram = 15½ grains
1 pound = 453.6 gms.
1 ounce = 28.4 gms.
1 drachm = 3.89 gms.
1 grain = 0.065 gms.

The Troy pounds and ounces are different but seldom used. For weights of organs, see **Normals.**

**Weil's Method.** For myelin sheaths (Weil, A., Arch. Neurol. & Psychiat., 1928, **20**, 392). Place celloidin sections of formalin fixed material (not yet mordanted) in 5% aq. potassium bichromate, 5 min. Wash twice in tap water. Stain for 15 min. at 45–50°C. in equal parts of 4% aq. iron alum and 1% aq. hematoxylin prepared from 10% sol. in abs. alc. at least 6 months old. Wash in tap water. Differentiate in 4% aq. iron alum until gray matter or degenerated areas become recognizable. Wash 3 times in tap water. Differentiate over white background to desired degree in: borax, 2.5 gm.; potassium ferricyanide, 12.5 gm.; aq. dest., 1000 cc. Dehydrate in 95% alc., abs. alc. and ether, clear in xylol and mount in balsam or clarite X.

If the differentiation in borax-ferricyanide mixture takes longer than 5 min. or in case the sections are over 30 $\mu$ thick repeat above from treatment with 5% bichromate to that with borax-ferricyanide. Then place sections in 0.25% aq. potassium permanganate, 10–30 sec. Wash in tap water. Destain until gray areas or areas of degeneration are colorless in: oxalic acid, 2.5 gm.; sodium bisulphite, 2.5 gm.; aq. dest., 1000 cc. Wash in tap water. Ammonia water (10 drops ammonia to 50 cc. aq. dest.), 30 sec. Wash 3 times in tap water. Dehydrate, clear and mount as before.

**Wetting Properties.** An interesting method for investigating the cell membrane is to measure its wetting properties. The Mudds (S., and E. B. H., J. Exp.Med., 1926, **43**, 127–142; J. Gen. Physiol., 1931, **14**, 733–751) have noticed the responses of cells to a film of oil advancing between slide and cover glass. Erythrocytes are easily wetted by the oil; whereas, when leucocytes are surrounded by the film of oil, the oil does not wet their surfaces but remains separated from them by thin films of saline solution. The Mudd's thought that this indicated that the surface of erythrocytes is lipoid and that of leucocytes protein. Danielli (Bourne, p. 78) has expressed the view that the surfaces of both cells are probably coated with protein, the erythrocytes with serum albumen and the leucocytes with serum globulin. The wetting technique has been employed in a considerable number of experiments. Dawson and Belkin, J. A. and M., Biol. Bull., 1929, **56**, 80–86 and Marsland, D., J. Cell. & Comp. Physiol., 1933, **4**, 9–33 worked with amebae and Chambers, R., Biol. Bull., 1935, **69**, 331, and Kopac, M. J. and Chambers, R., J. Cell. & Comp. Physiol., 1937, **9**, 331–361 with naked arbacia eggs. See **Cell Membranes.**

**Whole Mounts** of tissues which are fairly thick are often very useful. See **Blood Vessels, Cartilaginous Skeleton, Corrosion Preparations, Epidermis, Insects, Mammary Glands, Nerve Endings, Ossification,** etc.

**Wilder's Method** of silver impregnation for reticular fibers (Wilder, H. C., Am. J. Path., 1935, **11**, 817–819). Fix in 10% formalin, Zenker or formalin-Zenker. Treat paraffin, celloidin or frozen section in 0.25% aq. potassium permanganate or in 10% aq. phosphomolybdic acid for 1 min. Rinse in aq. dest. and transfer to hydrobromic acid (Merck's conc. 34%, 1 part; aq. dest., 3 parts) for 1 min. This can be omitted after phosphomolybdic acid. Wash in tap water and in aq. dest., then dip in 1% aq. uranium nitrate (sodium free) 5 sec. or less. Wash in aq. dest. 10–20 sec. and place in Foot's silver diamino hydroxide for 1 min. To make this: add 8.1% aq. $NH_4OH$ drop by drop to 5 cc. 10.2% aq. $AgNO_3$ until brown ppt. is just dissolved. Then add 5 cc. 3.1% aq. NaOH and sufficient $NH_4OH$ to just dissolve ppt. Make up to 50 cc. with aq. dest. Dip quickly in 95% alc. and reduce for 1 min. in: aq. dest., 50 cc.; 40% neutral formalin (neutralized with magnesium carbonate), 0.5 cc.; 1% aq. uranium nitrate, 1.5 cc. Wash in aq. dest. Tone in 1:500 gold chloride (Merck's reagent), 1 min. Rinse in aq. dest. and treat with 5% aq. sodium thiosulphate (hyposulphite), 1–2 min. Wash in tap water. Counterstain as desired, dehydrate, clear and mount in balsam. Reticular fibers black. Note author's figures of lymph nodes.

**Windaus,** see **Digitonine Reaction.**

**Wintergreen Oil** (methyl salicylate) is used in the **Spalteholz Method** of clearing.

**Woods Metal** is now largely replaced by celluloid in the making of corrosion preparations.

**Wool Orange 2G,** see **Orange G.**

**Wool Red,** see **Amaranth.**

**Wound Healing,** method for study *in vitro* (Bentley, F. H., J. Anat., 1935–36, **70**, 498–506).

**Wright's Blood Stain.** This is a compound stain of the **Romanowsky** type. The Commission Certified (C.C.) product is available. Dry the smear in air. Cover the area between the wax lines with stain measured by drops from a medicine dropper. After 1 min. add same volume aq. dest., shifting the slide a little from side to side so that it mixes fairly well. A green metallic looking scum forms on the surface. Leave 2 or 3 min. Too long staining produces a precipitate. It may be necessary to use for dilution instead of aq. dest. the McJunkin-Haden buffer. Wash in tap water 30 sec. or more until thin parts of smear become pink or yellow. Dry by blotting with smooth filter paper and examine directly without mounting in balsam and adding a cover glass. Usually excellent results are obtained. If however it is desired to employ buffered solutions especially for sections consult Petrunkevitch, A., Anat. Rec., 1937, **68**, 267–280 and Lillie, R. D., Stain Techn., 1941, **16**, 1–6. The other most used blood stain is that of Giemsa with its several modifications. Ehrlich's triacid stain is less used nowadays.

**Xanthene Dyes.** As the name implies they are derivatives of xanthene. They comprise many indicators and are classified as acridines, fluoran derivatives, phe-

nolphthalein, pyronins, quinolines, rhodamines, and sulfonphthaleins.

**Xanthin,** see **Phosphine.**

**Xanthoproteic Reaction.** Treat section with cold fuming nitric acid. After a few minutes the proteins become colored yellow. Then rinse and expose to ammonia vapor which changes the color to orange. Not specific for proteins because there is also a nitration of aromatic radicals of phenols, alkaloids, etc. The color is often faint but fairly sharp (Lison, p. 127). See also Bensleys (p. 126).

**XL Carmoisine 6R,** see **Chromotrope 2R.**

**Xylidine Ponceau 3RS,** see **Ponceau 2R.**

**Xyloidin,** see **Pyroxylin.**

**Yeasts,** vital staining of, see **Brilliant Purpurin R.**

**Yellow M,** see **Metanil Yellow.**

**X-ray Diffraction** method for investigating structure of nerve myelin sheath (Schmitt, F. O., Bear, R. S. and Palmer, K. J., J. Cell. & Comp. Physiol., 1941, 18, 31–42. See, also, **Historadiography.**

**Yaws.** *Treponema pertenue,* 18–20 μ long, 6–20 uniform spirals. Same technique as for **Treponema Pallida.**

**Zenker's Fluid.** Potassium bichromate, 2.5 gms.; mercuric chloride, (corrosive sublimate) 5 gms.; aq. dest., 100 cc.; glacial acetic acid, 5 cc. Because this mixture does not keep well make a stock fluid of say 2 liters by adding mercuric chloride to saturation in 5% potassium bichromate. It will do no harm if more than sufficient mercuric chloride is used and remains undissolved at the bottom of the bottle. The main point is to reach saturation. This will require several hours unless the mercuric chloride is dissolved in the aq. dest. with the aid of gentle heat before adding the bichromate which has been pulverized in a mortar to facilitate solution.

Immediately before use add 5% of glacial acetic acid. Fix tissues 24 hrs. and wash in running water about 12 hrs. Dehydrate and imbed in the usual way. Remove mercuric chloride from sections by Lugol's iodine solution 5–10 min. and wash out the iodine in alcohol before staining. This fluid is employed in techniques too numerous to mention. It is called for in case of **Mallory's Connective Tissue** stain and for demonstration of **Tendons, Purkinje Cells, Muscle, Fibrin, Hemofuscin,** etc.

**Zenker Less Acetic** is the stock solution without addition of acetic acid. This will serve as a fixative for mitochondria; because, since it does not contain acetic acid, they are not dissolved. It is, however, not recommended for mitochondria.

**Formalin-Zenker** or **Zenker-Formol** is a very useful fixative indeed. Helly's fluid is Zenker with 5% formalin in place of the 5% acetic acid. Maximow has used 10% formalin instead of 5%. It is added, like the acetic acid, just before use. The time of fixation, washing, etc. is the same as for Zenker's fluid.

**Ziehl's Carbol-Fuchsin** (as emended Soc. Am. Bact.): A. Basic fuchsin, 0.3 gm.; 95% ethyl alcohol, 10 cc.: B. Phenol, 5 gm.; aq. dest., 95 cc. Mix A and B. Much used for the staining of **Acid Fast Bacilli.**

**Zinc.** Mendel and Bradley's Method (L. B. and H. C., Am. J. Physiol., 1905, 14, 313–327). Treat paraffin sections with 10% aq. sodium nitroprussate for 15 min. at 50°C. Wash carefully in running water. Add cover glass. Introduce under it one drop potassium sulphide solution which causes an intense purple color (Lison, p. 98).

**Zinc Chloride,** as substitute for mercuric chloride in Zenker's fluid (Russell, W. O., J. Techn. Meth. & Bull. Int. Assoc. Med. Museums, 1941, 21, 47).

**Zweibaum's Fixative.** Add 1 part 2% aq. osmic acid to 7 parts 3% aq. potassium bichromate, 6 cc., 2% chromic acid, 3 cc. and aq. dest., 5 cc. See **Sudan Black B.**

**Zymogen** is substance within cells that produces an enzyme (G. zymē, leaven + gennāō, I produce). It is usually seen in the form of granules. These zymogen granules as they occur in the acinous cells of the pancreas, in the chief cells of the stomach, in the serous (or zymogenic cells of the salivary glands and in other situations can be well stained with Bensley's **Neutral Gentian** or **Bowie's Ethyl Violet-Biebrich Scarlet.** They can also be readily studied in living cells and their behavior noted as material is discharged from the cells into the lumina of the acini by a method elaborated by Covell, W. P., Anat. Rec., 1928, 40, 213–223. The technique consists of carefully mounting the pancreas of a living mouse in such a way that the circulation continues and the influence of pilocarpine can be observed.

**Zymonema Dermatitidis,** see **Blastomycosis.**